

BIOLOGIC DUOFLOW CHROMATOGRAPHY SYSTEM

INSTRUCTION MANUAL

BIO-RAD

SAFETY

**Caution/Warning**

Disconnect power to any BioLogic DuoFlow system component before servicing. No user-serviceable parts are inside any component. Refer servicing to Bio-Rad service personnel. Call Bio-Rad Technical Support Chromatography Group at 1-800-4-BioRad.

BioLogic DuoFlow systems are certified to meet the I.E.C. 1010-1* standard for safety of laboratory equipment. Certified products are safe to use when operated in accordance with the instruction manual. This safety certification does not extend to other chromatography equipment or accessories not I.E.C. 1010 certified, even when connected to a BioLogic DuoFlow system.

This instrument is intended for laboratory applications only. This instrument should not be modified or altered in any way. Alteration of this instrument will void the manufacturer's warranty, void the I.E.C. 1010 certification, and create a potential safety hazard for the user.

BioLogic DuoFlow systems conform to the class A standards for Electromagnetic Emissions, intended for laboratory equipment applications. It is possible that emissions from this product may interfere with some sensitive appliances when placed nearby or on the same circuit as those appliances. The user should be aware of this potential and take appropriate measures to avoid interference.

Bio-Rad is not responsible for any injury or damage caused by the use of this instrument for purposes other than for which it is intended or by modifications of the instrument not performed by Bio-Rad or an authorized agent.

The BioLogic USB Bitbus Communicator complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) this device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

The USB Bitbus Communicator has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the device is operated in a commercial environment. This device generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this device in a residential area is likely to cause harmful interference, in which case the user will be required to correct the interference at his own expense.

*I.E.C. 1010 is an internationally accepted electrical safety standard for laboratory instruments.

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1.0 INTRODUCTION

1.1 OVERVIEW

The BioLogic DuoFlow chromatography system is specifically designed for the high resolution purification of proteins, peptides, and other biomolecules where recovery of biological activity is of primary concern. The DuoFlow F10 pumphead operates at up to 20 ml/min and 3500 psi when used with the Maximizer. The DuoFlow F40 pumphead operates at up to 80 ml/min and 1000 psi when used with the Maximizer.

The BioLogic DuoFlow system software provides an easy-to-use graphic interface and menu-driven software for manual operation, system setup, method editing, and run operations. The system software may be run on any PC running Microsoft® Windows® 2000.

The flexible control architecture allows the seamless integration of a wide variety of configurations with other Bio-Rad and non-Bio-Rad components to meet your purification requirements.

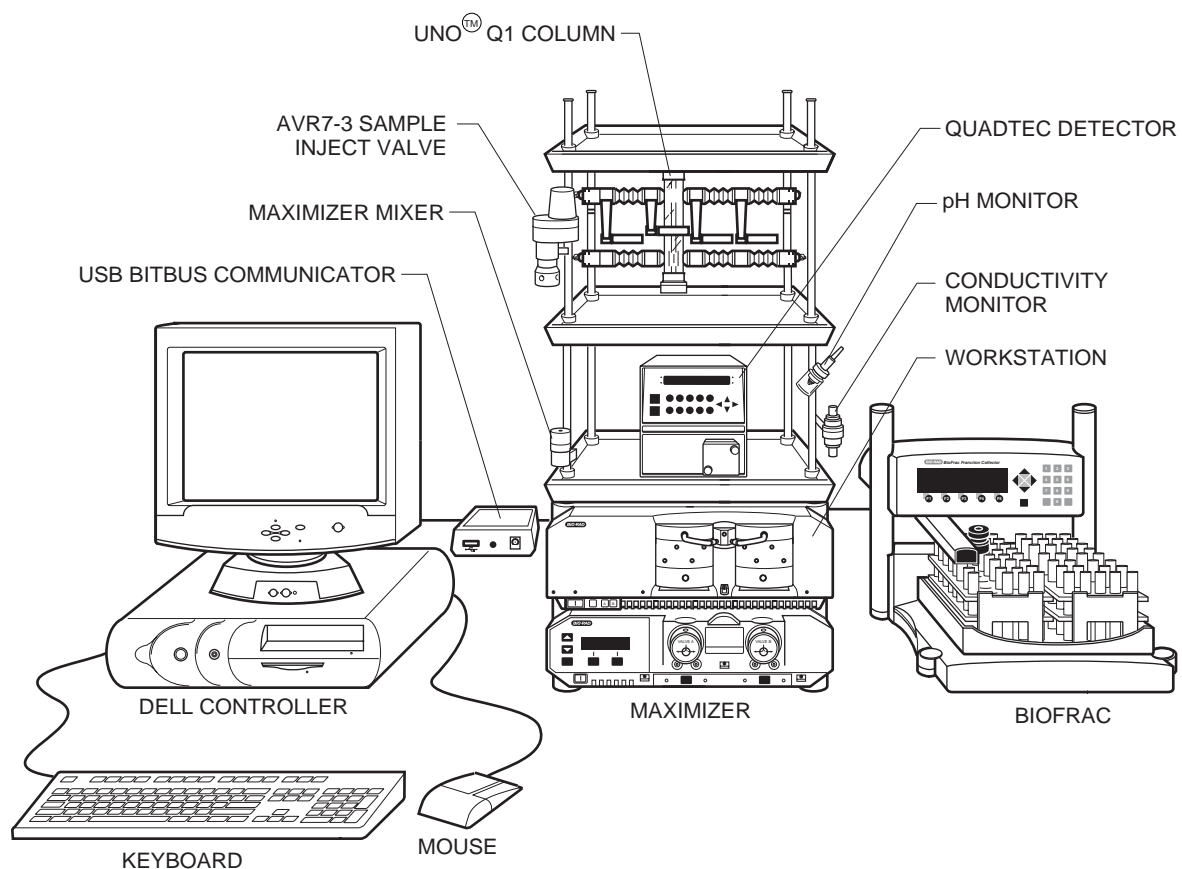


Figure 1-1. BioLogic DuoFlow System

1.2 FEATURES

BioLogic DuoFlow systems provide the following features:

- **Flexibility of setup.** A space saving modular design that is stackable enables configuring the system to meet your exact needs and fit into your desired bench space. Trays and vertical bars are moveable and removable. Horizontal bars can be placed in the optimal position for valves, columns, detectors, etc. The system fits easily into a cold box.
- **Modular** components provide an easy **upgrade path** to fit all applications and financial requirements. For example, the DuoFlow Basic system may be purchased and upgraded to a Pathfinder system as needed. (See Section 1.4 for a description of the DuoFlow systems and upgrades.)
- **Intuitive, user-friendly software programming.** Users become experts in only a few runs.
 - Four easy steps to set up new devices/instruments, create a new method, and start to run samples:
 - Step 1. Browser, to enter new user name and method name.
 - Step 2. Setup, to specify decides required for the method.
 - Step 3. Protocol, to enter sequential method steps.
 - Step 4. Run, to inject sample and view real time chromatogram.
 - Two easy steps, if you want to run a sample using a current Setup and Protocol:
 - Step 1. Browser, to select user and method.
 - Step 2. Run, to inject sample and view real time chromatogram.
- **On screen Help.** Easily accessed extensive, detailed explanations and troubleshooting guide.
- **USB Bitbus Communicator.** Bitbus communication allows users to connect their own USB compatible PC computer running Windows® 2000 and the DuoFlow version 4.0 or higher software. Bio-Rad provides a Dell computer running Windows® 2000 and the DuoFlow software version 4.0 or higher software with each DuoFlow system.
- **Buffer Blending** for pH methods development applications. The Maximizer add-on component provides this additional capability to the DuoFlow system.
- **pH Monitor.** The optional in-line BioLogic pH monitor allows direct monitoring of pH conditions during a run and comes with the Maximizer. It has a Calomel sealed electrode with both pH and reference electrodes in a single body. The sealed reference design eliminates the need to add electrolyte solutions and minimizes reference dry out. The electrode is fully compatible with buffers, like Tris, that may not be compatible with Ag/AgCl (silver/silver chloride) containing electrodes. It has a PEEK biocompatible flow cell.
- **Flow Rate Flexibility.** The Workstation has pumpheads that permit higher or lower flow rates. The DuoFlow system may be equipped with F10 pumpheads for slower flow rates from 0.01 ml/min, or F40 pumpheads for higher flow rates to 80 ml/min, when used with the Maximizer.
- **Detection Flexibility**
 - UV detector with fixed 254 nm and 280 nm filters, long life mercury lamp, and additional drop-in expansion filters available.
 - UV detector can be expanded to 214 nm filter with zinc lamp.
 - QuadTec UV/Vis detector analyzes samples simultaneously at 4 different wavelengths from 190-370 nm with a deuterium lamp or 370-790 with a halogen lamp.
 - Third party detectors, such as refractive index or fluorescent, may be utilized with our DuoFlow systems via a SIM or Maximizer.
- **Conductivity Monitor** monitors salt concentration to assure reliable gradient formation and pump functions.

- **Multiple Valve Capabilities**
 - Workstation provides connection for 3 low pressure and 3 high pressure valves.
 - Workstation with the addition of the Maximizer doubles the capacity to 6 low pressure and 6 high pressure valves.
- **Starter Kit and UNO Q1 Anion Exchange Column** provides the reagents, protein sample, and column. Easy to follow tutorial style instructions enable users to learn how to run a sample under known conditions.
- **IQ/OQ Protocols.** Validation protocols are available or can be performed by certified Bio-Rad service engineers.

1.3 UNPACKING

When you receive the BioLogic DuoFlow system, carefully inspect the shipping containers for any damage which may have occurred in shipping. Severe damage to a container may indicate damage to its contents. If you suspect damage to the contents, immediately file a claim with the carrier in accordance with their instructions before contacting Bio-Rad Laboratories.



Caution

Lift items from the bottom as you remove them from their containers!

Open each of the shipping cartons and lift the contents out of its packing. Check the contents of each box against the supplied packing list. Remove the plastic bag from each unit and inspect the unit for external damage. If any part is missing or damaged, contact Bio-Rad Laboratories immediately.

Bio-Rad ships DuoFlow systems in a number of different configurations, each with its own catalog number. These systems are described in the following table. Because of its modular design, any of these systems can be upgraded any time simply by adding system options.

1.4 SYSTEM CONFIGURATIONS

The BioLogic DuoFlow is available in the following system configurations. Each system configuration is identified by its name, its standard components and devices, and optional components and devices that may be ordered separately for use with the system.

Catalog Number	BioLogic DuoFlow System Guide	F10 Workstation	F10 Pump Kit	F40 Workstation	F40 Pump Kit	BioFrac Fraction Collector	QuadTec UV/Vis Detector	Maximizer (incl pH)	pH Monitor
	S = Standard U = Upgradable								
760-0037 760-0036 760-0038	DuoFlow Basic System 100/120 V DuoFlow Basic System Japan and Korea DuoFlow Basic System 220/240 V 10 ml/min flow rate to 3500 psi 254/280 nm detection	S	S	U	U	U	U	U	U
760-0047 760-0046 760-0048	DuoFlow Standard System 100/120 V DuoFlow Standard System Japan/Korea DuoFlow Standard System 220/240 V 10 ml/min flow rate to 3500 psi 254/280 nm detection Fraction collection	S	S	U	U	S	U	U	U
760-1137 760-1136 760-1148	DuoFlow QuadTec Basic System 100/120 V DuoFlow QuadTec Basic System Japan/Korea DuoFlow QuadTec Basic System 220/240 V 10 ml/min flow rate to 3500 psi UV/Vis detection with 4 simultaneous wavelengths	S	S	U	U	U	S	U	U
760-1147 760-1146 760-1148	DuoFlow QuadTec Standard System 100/120 V DuoFlow QuadTec Standard System Japan/Korea DuoFlow QuadTec Standard System 220/240 V 10 ml/min flow rate to 3500 psi UV/Vis detection with 4 simultaneous wavelengths Fraction collection	S	S	U	U	S	S	U	U
760-2237 760-2236 760-2238	DuoFlow Maximizer 20 System 100/120 V DuoFlow Maximizer 20 System Japan/Korea DuoFlow Maximizer 20 System 220/240 V 20 ml/min flow rate to 1000 psi 254/280 nm detection Buffer blending Fraction collection	S	S	U	U	S	U	S	S
760-2247 760-2246 760-2248	DuoFlow Maximizer 80 System 100/120V DuoFlow Maximizer 80 System Japan/Korea DuoFlow Maximizer 80 System 220/240 V 80 ml/min flow rate to 1000 psi 254/280 nm detector Buffer blending pH monitoring Fraction collection	U	U	S	S	S	U	S	S
760-2257 760-2256 760-2258	DuoFlow Pathfinder 20 System 100/120 V DuoFlow Pathfinder 20 System Japan/Korea DuoFlow Pathfinder 20 System 220/240 V 20 ml/min flow rate to 1000 psi UV/Vis detection with 4 simultaneous wavelengths Buffer blending pH monitoring Fraction collection	S	S	U	U	S	S	S	S
760-2267 760-2266 760-2268	DuoFlow Pathfinder 80 System 100/120 V DuoFlow Pathfinder 80 System Japan/Korea DuoFlow Pathfinder 80 System 220/240 V 80 ml/min flow rate to 1000 psi UV/Vis detection with 4 simultaneous wavelengths Buffer blending pH monitoring Fraction collection	U	U	S	S	S	S	S	S

1.5 QUICK START PROCEDURE

The following describes the general procedure to run a sample with your DuoFlow system.

1. Install the relevant devices and instruments. Refer to figures 3-4 and 5-5 for electrical connections and figures 4-1 and 4-3 for plumbing connections.
2. Flush all plumbing with DDI H₂O to ensure that the DuoFlow system is clean and free of air. Prime the pumps with your starting buffer or mobile phase. Refer to Chapter 4 for more information.
3. Equilibrate the system and your column with the starting buffer or mobile phase via the Manual screen options (see pg 7-2). Use the Manual screen to set the Workstation pump pressure high and low limit settings. The high limit setting should be the maximum backpressure acceptable for your column.
4. Select Browser from the Tool bar. In the Browser screen you will enter a username and name your new method. Refer to page 6-1 for more information on the Browser screen.
 - Select the **Browser** icon from the tool bar menu.
 - Select the **NEW** icon from the upper left side of the Browser screen.
 - Select **NEW** and enter your desired username in the dialog box.
 - Click on the Project icon for your username.
 - Select **NEW** and **NEW METHOD**. Enter your method name (or use default Method 1). Click **OK** to proceed to the hardware Setup screen.
5. In the Setup screen select the devices and instruments you will use for this method. You may name and save this particular configuration so that it can be recalled to be used with a different method protocol. To do this, select **Save Setup** under the File menu and enter the desired name. For more information on the Setup screen, refer to page 7-5.
6. Select Protocol from the tool bar. In the Protocol screen create your method. For more information on this screen, refer to page 7-9. If you are running an ion exchange experiment, you might want to begin your method as follows by selecting from the tool bar icons along the left side of the screen:
 - Select the fraction collector icon on the side bar menu. Select your fraction collection technique (e.g. collect all, threshold, etc.) and the volume of sample you wish to collect. After entering your entire method, return to the fraction collector and you will find the number of tubes required for collection has been calculated.
 - Add an Isocratic flow step and enter a flow rate and the amount of time required to equilibrate your column. The flow rate you select here will automatically appear in any subsequent steps, but can be changed at any time. Refer to page 7-10 for more information.
 - Add a Zero baseline step to zero your selected detector before injecting your sample.
 - Add an Inject sample step to select the sample volume to be injected. If you are using an AVR7-3 inject valve, select static loop. Your options will vary according to the type of injection method you chose when you set up your hardware. Refer to page 7-11 for more information.
 - Add a Linear Gradient step to select buffers or solvents to mix a gradient that will elute your sample. Refer to page 7-13 for mor information.
 - Add an Isocratic flow step with 100% of the final buffer or solvent in the linear gradient. This assures that all sample is removed from the column.
 - Add an Isocratic flow step with 100% of the starting buffer or solvent to ensure that your system is ready to inject the next sample.
 - You may also wish to include a Hold step in your method which will hold your method while the pumps continue working. This is useful if you wish to hold your gradient at some point for a specific amount of time. You may also enter a continue time to stop the hold and continue the method. See Chapter 7 for more information. For more information refer tp page 7-16.
7. Use the Run screen to run your method. Select the **RUN** icon from the tool bar menu to bring up the Run screen. For more information on this screen, refer to Chapter 7.

1.6 PROGRAMMING THE MAXIMIZER

The Maximizer valve system added to the DuoFlow system enables automated buffer blending protocols, doubles the flow rate of the Workstation pumps, and doubles the number of valves that may be connected to the DuoFlow system. Following are the basic procedures for running a buffer blending method. Refer to pages 2-11; 3-5; 4-3,4; 7-6; 11-13 for more information regarding installation and maintenance of your Maximizer system.

Overview to run a buffer blending method:

Manual Screen

In the Manual screen select the buffer blending icon from the tool bar and select your desired buffer from the menus of available buffers. Prepare your solutions according to the recipes provided and place them on the correct valve position of the Maximizer acid at A1, base at A2, salt at B1, and water at B2. Assure that the "Use pH correction" box is not checked. In the Maximizer*Pump control panel enter the pH value you are using (Desired), the salt composition in %B and you desired flow rate, press run. After the system has equilibrated collect some effluent and measure the pH (Observe pH at 0% B). These values will be entered into the buffer blender in Setup screen when you run you method.

Browser

Select a User name or enter a new name. Select -> New -> Method and enter a method name or accept the default of Method.1.

Setup

Select and enter the relevant information as prompted by the software for the pumps, valves, detectors, etc., in use for the method. Select Buffer Blender and enter the Desired pH and Observed pH values obtained from the Manual run. Assure that "Use pH correction", box is checked.

Protocol

Enter desired method steps. When you select Isocratic Flow and Linear Gradient your chosen buffer system will appear.

Load your sample, Start and Run

Solution Preparation

The predefined buffer recipes provided for use with the Maximizer have been formulated to give optimum accuracy near the pKa of the buffer. Buffer solutions should always be made with reagent grade materials and filtered or degassed.

pH Measurement and Corrections

Maximizer compensates for changes in temperature and ionic strength that affect the pH of a buffer. A sensor in the conductivity flow cell monitors temperature when connected to the Maximizer.

Single Point Correction (best for isocratic applications)

Accuracy can be improved by applying a single or double point pH correction.

1. Run your buffers in Manual mode and measure the pH of the eluant after the system has calibrated. When selecting buffers from Buffer Blending in the Manual screen assure that the pH correction box is not checked.
2. In the Browser select your desired User and method.
3. In the Setup screen select Buffer Blender and select the buffer in use.
4. Place a check in the "use pH Correction" box and assure that the "use two point correction" option is not selected.
5. Enter the pH value that was measured in step 1 in the "observed at 0% B" box and the desired pH in the "Desired" box and press OK. The software will calculate the correction.

Two Point Correction

1. Follow the same protocol for single point correction as defined in step 1, however set the salt composition to 0% B, press run.
2. After the system has equilibrated collect some low salt effluent to use to measure the pH.
3. Set the salt composition to the maximum to be used in the method. Take the pH electrode out of line as it can take a long time to equilibrate the electrode after exposure to high salt.
4. Press run, after the system has calibrated collect some high salt effluent to be used to measure the pH.
5. Measure the pH of the low and high salt solutions collected in steps 2 and 4.
6. In the Browser screen select your desired User and method.
7. In the Setup screen select Buffer Blender and select the buffer in use.
8. Place a check in the "use pH Correction" box and the "use two point correction" option.
9. Enter the low salt and high salt pH values collected in step 5 in the observed and the %B that was used to collect the high salt pH.
10. Enter the desired pH in "Desired."
11. The software will calculate the correction.

High flow rate chromatography without buffer blending

An option is available allowing you to run high flow rates, up to 80 ml/min with F40 pumpheads, without doing buffer blending method protocols as follows:

1. In the Browser select User and method you wish to run.
2. In the Setup screen double click on the Buffer Blender icon, choose "High Flow Non-Blending" from the pH recipe list.
3. Prepare the buffer that is placed at valve A1 at a 1X concentration and at the desired pH, plug the A2 port.
4. Prepare the buffer that is placed at valve B2, probably high salt, at 2X concentration and at the desired pH. Place a plug in port B2.
5. Prime the system and start your run.

2.0 DESCRIPTION OF SYSTEM COMPONENTS

The DuoFlow's modular design supports many types of system components, allowing for a wide variety of system configurations. This section discusses in detail the function of each component and its connection to the system.

- Dell PC Computer/Controller and USB Bitbus Communicator: see Section 2.1.
- Workstation: see Section 2.2.
- Maximizer: see Section 2.3.
- Mixers, which consist of the MX-1 mixer and the Maximizer mixer: see Section 2.4.
- Detection Systems, which consist of the UV detector, Conductivity monitor, and QuadTec detector: see Section 2.5.
- Valves, which consist of the AVR7-3, AVR9-8, SV5-4, and SVT3-2 valves: see Section 2.6.
- Fraction Collectors, which include the BioFrac, Model 2110, and Model 2128: see Section 2.7.
- Sample Loading Options, including the DynaLoop, EP-1 Econo pump, and EGP Econo Gradient Pump: see Section 2.8.
- System Peripherals include the SIM, pH monitor, and universal power supply: see Section 2.9.
- Columns and Column Fittings: see Section 2.10.

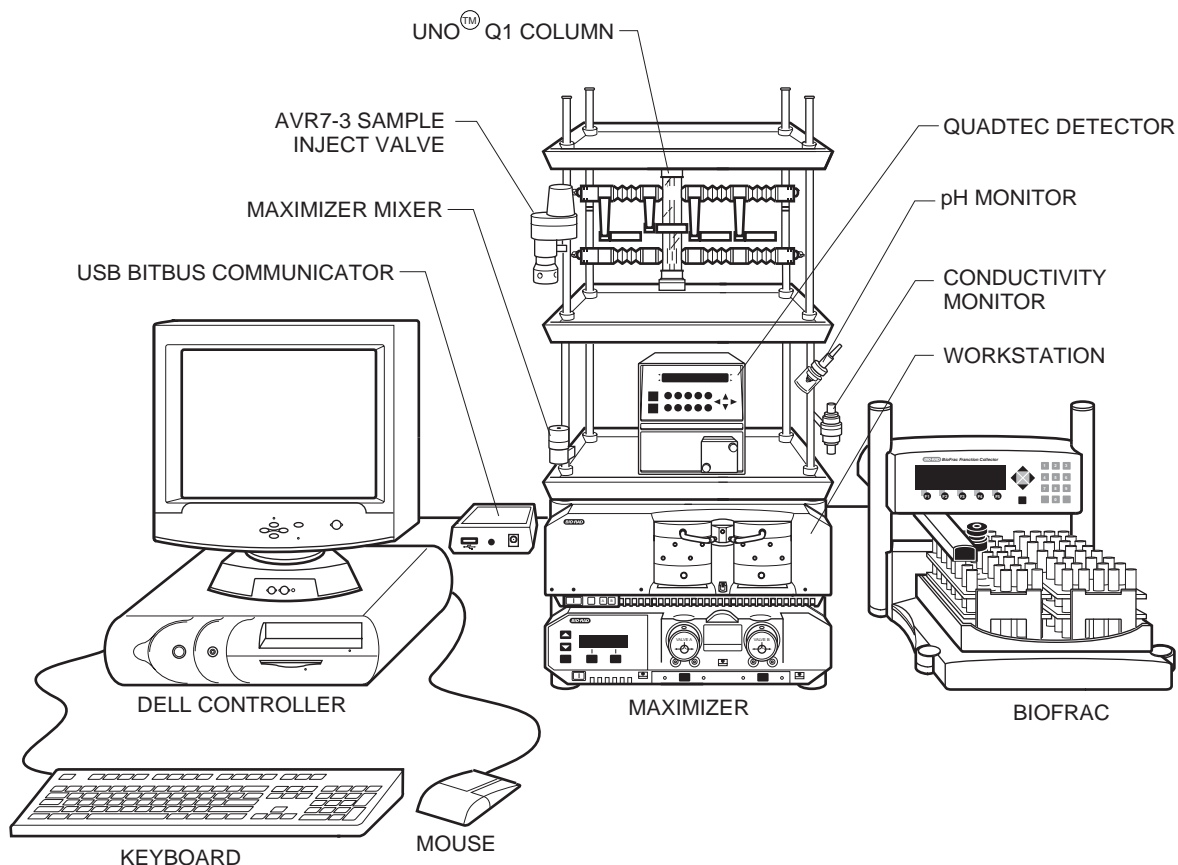


Figure 2-1. Pathfinder DuoFlow System Components

2.1 CONTROLLER AND USB BITBUS COMMUNICATOR

The DuoFlow systems are controlled by a PC computer, referred to throughout this document as the Controller. The Dell Controller available from Bio-Rad includes a color display monitor, a keyboard, a mouse device, a CD-ROM drive, and a floppy disk drive.

The Controller communicates with the workstation and other external devices via its USB connector. These devices include the DuoFlow system components, which communicate via the instrument bus connectors. The USB Bitbus Communicator serves as the link between the Controller's USB connection and the DuoFlow's Instrument bus connections. This link allows the Controller to control the Maximizer and Workstation, as well as any instruments connected to the Maximizer or Workstation, such as automatic valves, UV detector and conductivity monitor, and peripheral instruments such as the BioFrac fraction collector, the QuadTec UV/Vis detector, the Model EP-1 Econo pump, the Econo Gradient Pump (EGP), and Signal Import Modules (SIM) for additional devices.

2.1.1 Controller

The Controller runs BioLogic DuoFlow version 4.0 or higher system control software on Windows® 2000 or higher operating system. From the Controller you can set up and run methods, perform simple data analysis, and store method and run data. The following tables show the key features on the Dell computer provided by Bio-Rad.

Table 2-1.
Front View of the Dell PC Computer as the DuoFlow Controller

Feature	Description
Power Switch	Turns on/off the Controller and monitor.
Floppy Disk Drive	To backup methods to - and restore methods from - a floppy disk. Press the button next to the drive slot to manually eject a floppy disk from the drive.

Table 2-1. (continued)
Front View of a Dell PC Computer as the DuoFlow Controller





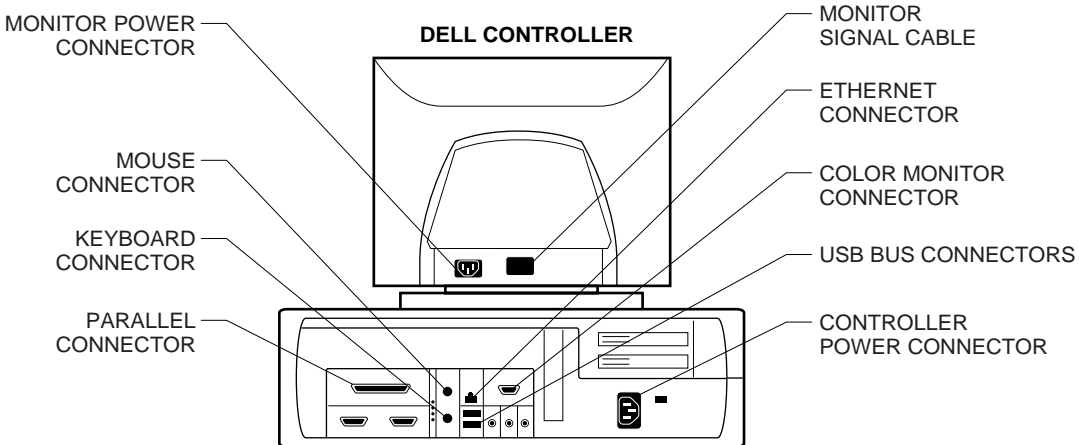




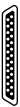

Feature	Description
CD ROM Drive	To load updates of the BioLogic DuoFlow operating software. To open the drive, press the button on the front of the drive.
Keyboard, Mouse, & Function Keys    	<p>The keyboard and mouse interface devices control the system. They are standard PC compatible input devices. The keyboard includes the following special function keys:</p> <p>Hold until Keypress: To start a method that is on Hold during a run when a method includes a “Hold until Keypress” step.</p> <p>Help: Displays the Help menu for the currently displayed screen.</p> <p>Esc: Functions as an alternative to the Cancel selection in a Dialog box.</p> <p>Alt: Some system commands can be executed either by selecting them from a drop-down menu or by holding down the Alt key and then pressing the appropriate character key.</p>
USB Connectors	These connect to the USB Bitbus Communicator, which in turn connects to the Instrument bus, thereby allowing components in the BioLogic DuoFlow system to communicate with the Controller. Components connect to the Instrument bus in a “daisy-chain.” The presence of a component on the Instrument bus is recognized when it is switched on. Even when one component is switched off, another component “daisy-chained” to it can be controlled by the Controller.

Table 2-2.
Rear View of the Dell PC Computer as the DuoFlow Controller

	
Connector	Description
	USB connectors: To connect to the USB Bitbus Communicator, which connects to the Instrument bus, enabling components in the BioLogic DuoFlow system to communicate with the Controller. Components connect to the Instrument bus cable in a “daisy-chain.” The presence of a component on the Instrument bus is recognized when it is switched on. Even when one component is switched off, another component “daisy-chained” to it can be controlled by the Controller. There are two USB ports behind a door on the front of the Controller.
	Monitor connector: To connect the color monitor to the Controller.
	Keyboard connector: To connect the keyboard to the Controller.
	Mouse connector: To connect the mouse to the Controller.
	Parallel connector: Devices designed for connection to the parallel port include printers and external storage devices. Refer to Windows® 2000 and/or the device documentation for installation instructions. (Some printer drivers are pre-installed on the Controller.)
	Power connectors: To connect the power cable.

2.1.2 USB Bitbus Communicator

The USB Bitbus Communicator connects the Controller to the rest of the DuoFlow system. It allows instruments connected via the Instrument bus cable to communicate with the Controller USB bus.

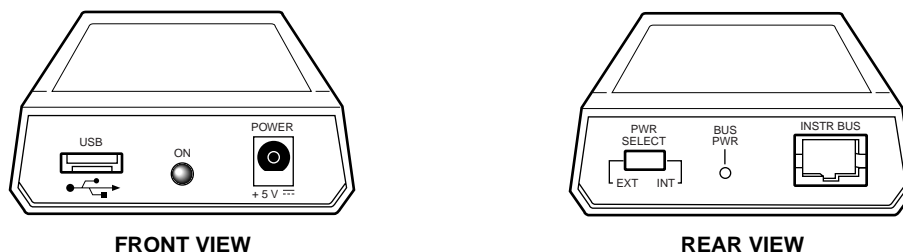


Figure 2-2. USB Bitbus Communicator

The following connectors are available from the front of the USB Bitbus Communicator:

- **USB connector:** The Controller communicates with other devices through its USB bus. Connects to the Controller USB bus connector.
- **ON LED:** Indicates there is power to the USB Bitbus Communicator.
- **Power connector:** Additional power is required when a Signal Import Module (SIM) is used with the DuoFlow system. A universal AC/DC inline adapter (catalog number 760-2084) supplies the power.

The following connectors are available from the back of the USB Bitbus Communicator:

- **Power select switch:** The USB Bitbus Communicator is powered directly from the controller via the USB cable, set this switch to **Internal**. The “BUS PWR” light on the rear panel does not illuminate. If a Signal Import Module (SIM) is to be used, set this switch to **External**. Power to the USB Bitbus Communicator will be via a universal AC/DC inline adaptor (catalog number 760-2034). The “BUS PWR” light on the rear panel will illuminate green.
- **Bus power LED:** Indicates connection to a SIM module by illuminating green.
- **Instrument bus connector:** To connect the USB Bitbus Communicator to the DuoFlow workstation.

The setup of the USB Bitbus Communicator is different if a SIM is to be used with the system. The differences are explained briefly below; see also Chapter 3, System Cabling.

- **USB Bitbus Communicator *without* a SIM:** In this configuration, the power adapter is not required and the Power Select switch on the USB Bitbus Communicator should be set to **Internal**.
- **USB Bitbus Communicator *with* a SIM:** In this configuration, the power adapter is required and the Power Select switch should be set to **External**.

2.2 WORKSTATION

The Workstation contains the following:

- Dual pumpheads, each consists of two biocompatible dual piston pumpheads. A built-in pressure transducer is located on the workstation at the pump outlet. The pressure transducer measures system pressure, which is displayed on the lower status bar of the software Manual and Run screens. Purge and Pause buttons (Purge A, Purge B, and Pause) are present on the front of the Workstation.

There are two types of pumpheads; the F10 and the F40. All DuoFlow systems have F10 pumpheads, except DuoFlow Maximizer 80 and DuoFlow Pathfinder 80 systems that have F40 pumps.

F10 and F40 pumphead kits are available to easily convert an F10 Workstation to F40 and vice versa.

Table 2-3.
F10 and F40 Pumphead Flow Rates

Pumphead	Flow Rate: Isocratic and Gradient Mode	Flow Rate with Maximizer: High Flow Non-blending	Flow Rate with Maximizer: Buffer Blending Mode
F10	0.01 - 10 ml/min 3500 psi (233 bar, 23 MPa)	0.02 - 20 ml/min 3500 psi (233 bar, 23 MPa)	0.5 - 20 ml/min 3500 psi (233 bar, 23 MPa)
F40	0.5 - 40 ml/min 1000 psi (66 bar, 6.6 MPa)	1.0 - 80 ml/min 1000 psi (66 bar, 6.6 MPa)	1.0 - 80 ml/min 1000 psi (66 bar, 6.6 MPa)

- The Workstation houses the control circuitry for the Workstation pumps, MX-1 mixer, UV detector, Conductivity monitor, and system valves (low pressure solenoid and automated high pressure inject and select valves). Connectors on the rear panel provide inputs for valves and detectors, as well as output to a chart recorder for UV and conductivity data at 1 V Full Scale and pen up/down, start/stop control.
If a Maximizer is being used, connect devices to it to connect the mixer, Conductivity monitor, and pH monitor to it, rather than to the Workstation.
- An AUX combicon connector used for (a) controlling fraction advances of the Model 2110 and generic fraction collectors, (b) receiving an open/closed signal from a device such as a manual inject valve, and (c) starting and stopping the Model EP-1 Econo pump for sample loading.
If a Maximizer is being used, connect these devices to its AUX connector, rather than to the Workstation.
- Power supply for the Workstation electronics as well as all devices connected to and controlled by the Workstation.
- Output power connectors to the UV lamp and a Model 1327 chart recorder.
- Two instrument bus (phone-type) connectors.

Table 2-4.
Workstation Front Panel Controls

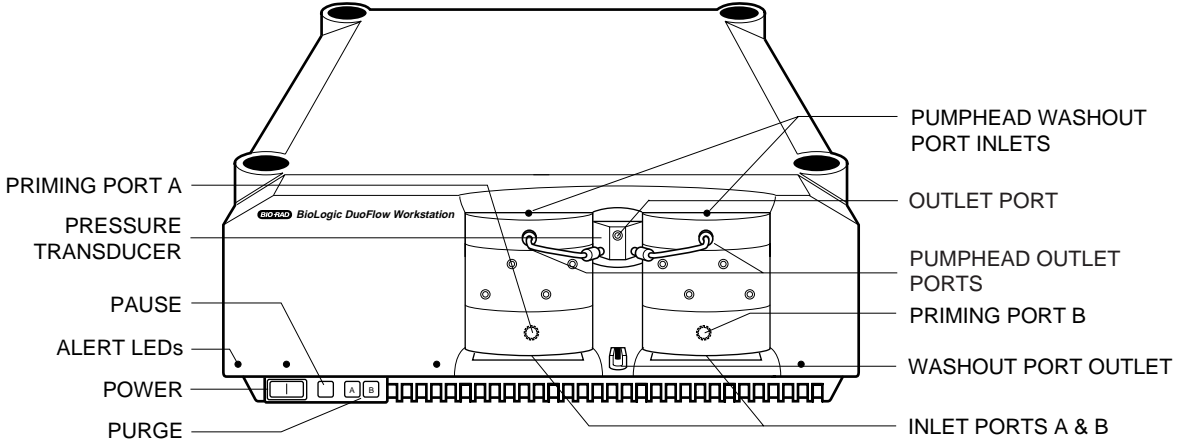
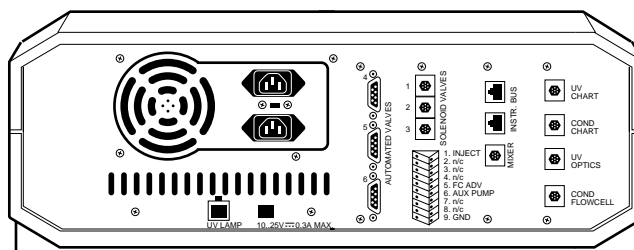
	
Feature	Description
Power Button	Turns power on/off to the Workstation and to the components connected to it.
Pause Button	<p>Stops the Workstation pumps and pauses a running method. When it is pressed, the status LEDs for pumps A and B change from green to flashing red. This button is useful in the event of last minute changes or corrections to a method. To resume the run,</p> <ul style="list-style-type: none"> • Press the button again, which restarts the pumps and the method, or • Press the Continue button in the Run screen, or • Press the START button in the Manual screen.
Purge A & B Buttons	<p>Before you press either purge button switch the AVR7-3 inject valve to the Purge position in the Manual screen, so that the column is not exposed to high pressures and flow rates.</p> <p>The purge buttons flush the tubing lines with the solution connected to pumps A and B. When the buttons are pressed, the green LEDs for pumps A and B flash. To stop the flow, press the purge buttons again and the LEDs go off. These buttons do not operate when the system is running a method.</p> <p>When the purge buttons are pressed, the pumpheads run at their (default) maximum flow (F10 purge rate is 10.0 ml/min or 20 ml/min when used with the Maximizer; F40 purge rate is 40 ml/min or 80 ml/min when used with the Maximizer.) To alter the default purge flow rate, select Manual Setup from the Options drop-down menu.</p>
Alert Light	<p>This LED serves two functions:</p> <ol style="list-style-type: none"> Constant red: Indicates that the pump has shut down due to a mechanical or electrical problem. This includes a shutdown due to a high or low pressure limit being exceeded. Pressure limits are set at the Manual screen. Flashing red: Occurs during a method run and indicates that a pre-programmed ALARM step has been reached. The system operator must respond before the method continues. Also, the Controller emits an audible tone. Constant green: Indicates the pump is running.

Table 2-4. (continued)
Workstation Front Panel Controls

Feature	Description
Plumbing Connections	<p>Ports on the front of the Workstation:</p> <ul style="list-style-type: none"> a. Pumphead Inlet ports: Buffer inlet lines attach to the bottom of each pumphead using standard 1/4-28 flat-bottom fittings. The pumphead inlet tubing is 1/8" (3.2 mm) OD, 0.062" (1.6 mm) ID Teflon tubing with flat bottom fittings which are supplied in the Fittings kit. b. Pumphead Outlet and Pressure Transducer Inlet and Outlet ports: All plumbing following the pumphead outlet ports uses standard 1/4-28 flat-bottom fittings and the following tubing: Orange PEEK tubing: 1/16" (1.6 mm) OD, 0.020" (0.51 mm) ID. Used with pressures up to 5000 psi. Green PEEK tubing: 1/16" (1.6 mm) OD, 0.030" (0.76 mm) ID. Used with pressures up to 3000 psi, usually for flow rates greater than 40 ml/min. c. Pumphead Priming ports: This port on each pumphead is used to prime the pump. The port accepts any size syringe with a luer fitting; a syringe is included in the Fittings kit. This draws buffer along the inlet line and to the pumphead. Twist the port counter-clockwise one turn to open it. d. Pumphead Washout ports: The port on top of each pumphead is used to rinse the piston to remove crystallized salts. It accepts any syringe with a luer fitting. A syringe for this purpose is included in the Fittings kit. The rinse output is the open trough between the pumpheads.

Table 2-5.
Workstation Rear Panel Connectors















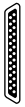
Connector	Description
	Solenoid Valves: To connect DuoFlow low pressure solenoid valves (SV5-4 and SVT3-2) to the system. If a Maximizer is in use, connect to its solenoid valve connectors before those on the Workstation.
	Automated Valves: To connect DuoFlow high pressure automated valves (AVR7-3 Inject and AVR9-8 Stream Select) to the system. If a Maximizer is in use, connect to its automated valve connectors before those on the Workstation.
	Cond Flowcell: To connect the Conductivity monitor flow cell to the system. If the Maximizer is being used, you must use its Cond Flowcell connector rather than the connector on the Workstation.
	UV Lamp: This specialized 6-pin square connector provides electrical power to the mercury or zinc lamp in the UV detector lamp housing. This connector is not available on the Maximizer.
	UV Optics: To connect the UV detector to the system.
	Mixer: To connect the mixer to the system. If the Maximizer is in use, connect to its mixer connector before those on the Workstation.
	10...25V --0.3A Max: Provides electrical power to the Model 1327 chart recorder.
	Cond Chart: For conductivity signal output to a single or dual pen chart recorder. An 8-pin mini-DIN to banana plug cable (System Cable 4) for connection to the Model 1327 chart recorder is available. Connect the red line to the positive (+) terminal and the black line to the negative (–) or ground terminal of channel 2 (CH2). The chart recorder should be set to 1 V full scale. If you are using the Model 1327 chart recorder, move all switches to the “green” settings.

Table 2-5. (continued)
Workstation Rear Panel Connectors

Conector	Description																				
	<p>UV Chart: For UV signal output to a single or dual pen chart recorder. When the Bio-Rad Model 1327 is used, chart recorder Pen Up/Down, Stop/Start commands, and event marks are sent from this connector.</p> <p>The Bio-Rad Model 1327 dual pen recorder needs an 8-pin mini-DIN to standard DIN cable (System Cable 2) and a mini-DIN to banana plugs cable (System Cable 4).</p> <p>Generic chart recorders require an 8-pin mini-DIN to breakout cable (System Cable 7), available from Bio-Rad.</p> <p>When a Signal Import Module signal replaces the standard BioLogic UV Detector, use System Cable 20 to control a Bio-Rad Model 1327 dual pen chart recorder.</p> <p>The chart recorder should be set to 1V.</p>																				
	<p>Power Cord: The grounded 3-prong connector inputs power to the Workstation and outputs power to any unit connected to the Workstation. The Workstation's input power cord should be plugged into a 3-prong grounded power outlet.</p>																				
	<p>Instr Bus: The RJ-45 modular phone connectors and the bus communication cables connect the Workstation to the other components in the system. The Instrument Bus handles all communications between the Controller and each of the components in the system. For example, the Instrument Bus connects the Workstation to the USB Bitbus Communicator, Maximizer, BioFrac fraction collector, or Econo Gradient Pump. Components can be connected in any order in the system.</p>																				
 <div style="display: inline-block; vertical-align: middle; margin-left: 10px;"> <p>AUX PORT</p> <p>1 INJECT 2 N/C 3 N/C 4 N/C 5 FC ADV 6 AUX PUMP 7 N/C 8 N/C 9 GND</p> </div>	<p>Aux: The 9-pin AUX PORT connects a variety of peripheral modules that cannot communicate with the DuoFlow Controller over the Instrument Bus. If the Maximizer is being used, use its AUX connector before the connector on the Workstation.</p> <table border="1"> <thead> <tr> <th>Pin #</th><th>Description</th></tr> </thead> <tbody> <tr> <td>1</td><td>Inject. A contact closure between pins 1 and 9 (GND) satisfies a Hold command which has been programmed in a method protocol.</td></tr> <tr> <td>2</td><td>n/c. No connection</td></tr> <tr> <td>3</td><td>n/c. No connection</td></tr> <tr> <td>4</td><td>n/c. No connection</td></tr> <tr> <td>5</td><td>FC Adv. Model 2110 and generic fraction collector Advance output.</td></tr> <tr> <td>6</td><td>AUX Pump. A Stop-Start command is sent to a pump (e.g., Bio-Rad EP-1).</td></tr> <tr> <td>7</td><td>n/c. No connection</td></tr> <tr> <td>8</td><td>n/c. No connection</td></tr> <tr> <td>9</td><td>GND. Ground</td></tr> </tbody> </table>	Pin #	Description	1	Inject. A contact closure between pins 1 and 9 (GND) satisfies a Hold command which has been programmed in a method protocol.	2	n/c. No connection	3	n/c. No connection	4	n/c. No connection	5	FC Adv. Model 2110 and generic fraction collector Advance output.	6	AUX Pump. A Stop-Start command is sent to a pump (e.g., Bio-Rad EP-1).	7	n/c. No connection	8	n/c. No connection	9	GND. Ground
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4	n/c. No connection																				
5	FC Adv. Model 2110 and generic fraction collector Advance output.																				
6	AUX Pump. A Stop-Start command is sent to a pump (e.g., Bio-Rad EP-1).																				
7	n/c. No connection																				
8	n/c. No connection																				
9	GND. Ground																				
	Reserved for internal Bio-Rad use.																				

2.3 MAXIMIZER

The Maximizer enables buffer blending applications, doubles pump flow rates, and doubles valving capacity to 6 low pressure valves and 6 high pressure valves. The Maximizer includes a separate Maximizer mixer (see Section 2.4.2) and pH monitor (see Section 2.5.4).

- Proportioning valves on the Maximizer blend water, salt, and the conjugate acid and base of a buffer to obtain a solution with a user-defined pH and salt concentration. One valve delivers an acid and base and the other valve delivers a salt and water.
- Pre-defined protocols are provided for virtually all common buffer systems used in chromatographic applications.

For example, after the user selects the desired pH for each separation, the software determines the required acid/base ratio and during the run compensates for changes in ionic strength and temperature. This provides tremendous convenience by enabling multiple unattended runs, each at a different buffer pH and salt concentration.

- When the Maximizer is set to local mode in the software Manual screen, it will not be under DuoFlow system control. In local mode the Maximizer front panel controls are accessible and can be used to prime the system, calibrate the pH and conductivity monitor, observe the status of each device, and control the position of each Maximizer valve.
- The Maximizer is designed to operate under normal laboratory and coldroom conditions (4° - 40° C) with all commonly used aqueous chromatographic buffers.

The Maximizer includes the following hardware and circuitry:

- Front panel: on/off switches for the two proportioning valves, two 3-port, two position proportioning valves for automated buffer blending; an LCD screen and membrane switches for controlling valve positions.
- Rear panel: connectors that (with the exception of the UV detector) duplicate the connectors on the Workstation. It is recommended that valves, peripheral devices, and flow cells be connected to the Maximizer rather than the Workstation unless more valving capability is needed.
- Display screen: this screen displays information and allows the user to change inlets and set points and cancel calibration.

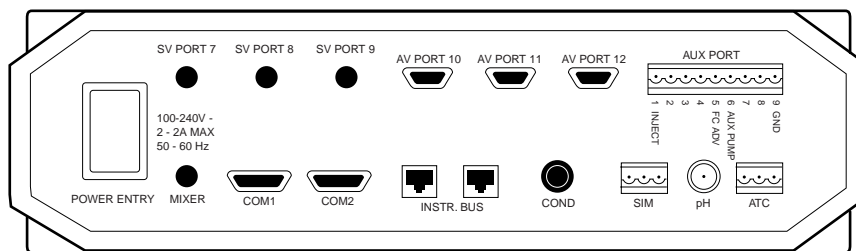
Table 2-6.
Maximizer Front Panel Controls

Feature	Description
Power On/Off	Controls power to the unit.
Valves A & B	The two proportioning valves may be manually controlled by the valve inlet select buttons below them or by the Controller. Each valve has two inlet ports and one outlet port. Valve A has inlet A1 for acid and A2 for base; valve B has inlet B1 for water and B2 for salt. The outlet ports on each valve connect directly to the Workstation pump inlet ports. Color coded tubing supplied with the Maximizer identifies each connector and solution.
Valve Inlet Select	To provide manual inlet control of valves A and B.
Valve Inlet Status LEDs	To indicate which valve port is open.
LCD Display	To display the status of the Maximizer. When the Maximizer is operated from its front panel controls, the LCD displays status and control information, including valve positions, pH and conductivity calibration information, and current temperature.

Table 2-6. (continued)
Maximizer Front Panel Controls

Feature	Description
Softkeys	For limited local operation of the Maximizer. This is discussed further in Table 2-8.
Enter and Arrow Keys	To operate the Maximizer in conjunction with the softkeys, as discussed above.

Table 2-7.
Maximizer Rear Panel Connectors



MAXIMIZER REAR







Connector	Description
	Solenoid Valves: To connect DuoFlow low pressure solenoid valves (SV5-4 and SVT3-2) to the system. If a Maximizer is in use, connect to its solenoid valve connectors before those on the Workstation.
	Automated Valves: To connect DuoFlow high pressure automated valves (AVR7-3 Inject and AVR9-8 Stream Select) to the system. If a Maximizer is in use, connect to its automated valve connectors before those on the Workstation.
 ATC	ATC: Reserved for future use.
	pH monitor: To connect the DuoFlow pH electrode. The pH monitor is described in greater detail in Section 2.5.4.
 SIM	SIM device: The Signal Import Module (SIM), enables connection of a detector or device that outputs an analog signal between -2.5 Volts to +2.5 Volts. Instruments that may be connected in this way could include a variable wavelength UV detector, a refractive index detector, or a fluorescence detector. The SIM digitizes the analog signal and transmits it to the BioLogic DuoFlow Controller. External SIM modules are discussed later in this chapter.
	Cond Flow cell: To connect the Conductivity flow cell. The Conductivity monitor flow cell must be connected to the Maximizer, to supply temperature information for pH compensation.

Table 2-7. (continued)
Maximizer Rear Panel Connectors





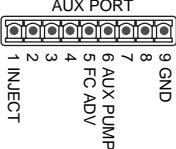

Connector	Description																				
	Instrument Bus: The RJ-45 modular phone connectors and their bus communication cables connect the Maximizer to the Controller and the Workstation (via the USB Bitbus Communicator). The Instrument Bus handles all communications between the Controller and each of the components in the system. Components can be connected in any order in the system.																				
	Com 1: To connect the QuadTec UV/Vis detector.																				
	Com 2: Reserved for future use.																				
	Mixer: To connect the mixer.																				
 <p>AUX PORT</p> <p>9 GND 8 7 6 AUX PUMP 5 FC ADV 4 3 2 1 INJECT</p>	<p>Aux: The 9-pin AUX port connects a variety of peripheral modules that cannot communicate with the DuoFlow Controller over the Instrument bus.</p> <table> <thead> <tr> <th>Pin #</th><th>Description</th></tr> </thead> <tbody> <tr> <td>1</td><td>Inject. A contact closure between pins 1 and 9 (GND) satisfies a Hold for inject command which has been programmed in a method protocol.</td></tr> <tr> <td>2</td><td>n/c. No connection</td></tr> <tr> <td>3</td><td>n/c. No connection</td></tr> <tr> <td>4</td><td>n/c. No connection</td></tr> <tr> <td>5</td><td>FC Adv. Model 2110 and generic fraction collector Advance output.</td></tr> <tr> <td>6</td><td>AUX Pump. A Stop-Start command is sent to a pump (e.g., Bio-Rad EP-1 pump).</td></tr> <tr> <td>7</td><td>n/c. No connection</td></tr> <tr> <td>8</td><td>n/c. No connection</td></tr> <tr> <td>9</td><td>GND. Ground</td></tr> </tbody> </table>	Pin #	Description	1	Inject. A contact closure between pins 1 and 9 (GND) satisfies a Hold for inject command which has been programmed in a method protocol.	2	n/c. No connection	3	n/c. No connection	4	n/c. No connection	5	FC Adv. Model 2110 and generic fraction collector Advance output.	6	AUX Pump. A Stop-Start command is sent to a pump (e.g., Bio-Rad EP-1 pump).	7	n/c. No connection	8	n/c. No connection	9	GND. Ground
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7	n/c. No connection																				
8	n/c. No connection																				
9	GND. Ground																				
	Power Cord: The grounded 3-prong connector inputs power to the Workstation and outputs power to any unit connected to the Workstation. The Workstation's input power cord should be plugged into a 3-prong grounded power outlet.																				

Table 2-8.
Maximizer Screens

Screen	Function and Description on Maximizer Faceplate in Local Mode
<div> BLEND A POS 0 PREV NEXT </div> <div> BLEND B POS 1 PREV NEXT </div>	<p>Inlet Selection</p> <p>Arrow buttons switch between Inlets A1(0) and A2(1). PREV changes to previous screen. NEXT changes to next screen.</p> <p>Arrow buttons switch between Inlets B1(0) and B2(1).</p>
<div> <Valve><Port> POS 0 PREV NEXT </div>	<p>Valve Control</p> <p>Arrow buttons select a port <Port> on valve <Valve>. Up to six valves may be displayed including three motorized (AVR7-3, AVR9-8) and three solenoid (SVT3-2, SV5-4) valves. ENTER accepts the change and moves the valve to the new position.</p>
<div> SIM 0.000 Volts PREV NEXT </div> <div> CAL SIM? ENT=SET PREV NEXT </div> <div> CAL SIM? ENT=SET PREV set NEXT </div> <div> CAL SIM? ENT=SET PREV reset NEXT </div>	<p>SIM Calibration</p> <p>Displays the current Maximizer SIM voltage.</p> <p>Used in conjunction with a 1 Volt calibration source. Pressing ENTER sets the current voltage reading to 1 V. Pressing the UP arrow resets the calibration to the factory setting.</p> <p>After ENTER is pressed, the display responds with “SET” to show that the calibration was successful.</p> <p>After the UP arrow is pressed, the display responds with “RESET” to show that the calibration was reset.</p>
<div> pH 6.00 22.6 C PREV NEXT </div> <div> CAL pH? ENTER = Y PREV NEXT </div>	<p>pH Calibration</p> <p>Displays the current pH and temperature.</p> <p>ENTER causes the Maximizer to enter pH calibration mode.</p>

Table 2-8. (continued)
Maximizer Screens

Screen	Function and Description
<div>1ST pH PT. _4.00 CURSOR CANCEL</div> <div>1ST: 35.22 mV CANCEL SET</div> <div>2ND pH PT. _7.00 CURSOR CANCEL</div> <div>2ND: 35.22 mV CANCEL SET</div>	<p>pH Calibration (continued)</p> <p>CURSOR moves the cursor to the next digit of the pH set point. UP/DOWN arrows adjust the pH set point. CANCEL aborts the calibration. ENTER accepts the pH value of the first calibration buffer and moves to the next screen.</p> <p>Display of the current pH voltage for monitoring probe equilibration. SET or ENTER sets the first calibration point. CANCEL aborts the calibration.</p> <p>CURSOR moves the cursor to the next digit of the pH set point. UP/DOWN arrows adjust the pH set point. CANCEL aborts the calibration. ENTER accepts the pH value of the second calibration buffer and moves to the next screen.</p> <p>Displays the current pH voltage for monitoring probe equilibration. SET or ENTER sets the second calibration point and exits pH calibration mode. CANCEL aborts the calibration.</p>
<div>COND. 0.86 PREV NEXT</div> <div>SET CELL C? ENT=Y PREV NEXT</div> <div>NEW CELL C: 43.00 CURSOR CANCEL</div>	<p>Conductivity Calibration</p> <p>Displays the current conductivity reading (mS/cm).</p> <p>ENTER causes the Maximizer to enter calibration mode.</p> <p>CURSOR moves the cursor to the next digit of the calibration constant. ENTER accepts the conductivity cell constant, calibrates the conductivity meter, and exits calibration mode. CANCEL aborts the calibration.</p>
<div>BEEPER? ENTER=Y PREV NEXT</div> <div>VOLUME: UP/DOWN ENTER TO FINISH</div>	<p>Beeper</p> <p>ENTER causes the Maximizer to enter volume adjustment mode.</p> <p>The UP and DOWN arrows adjust the volume of the alarm.</p>
<div>MAXIMIZER V3.xx NEXT</div>	<p>Firmware Version</p> <p>Displays the current version of the Maximizer firmware.</p>

2.4 MIXERS

Bio-Rad's DuoFlow mixers improve gradient quality by mixing the output from the DuoFlow Workstation pumps. There are two PEEK biocompatible mixers for DuoFlow systems.

- MX-1 mixer: A low volume mixer for use with a DuoFlow system **not** equipped with the Maximizer.
- Maximizer mixer: A large volume mixer for use with the Maximizer.

The mixer cable plugs into the connector labeled **Mixer** on the rear of the Maximizer or Workstation. If you will be using the MX-1 mixer, connect it to the Workstation; if you will be using the Maximizer mixer, connect it to the Maximizer. The mixers have two inlet ports; seal the unused port with the plug provided.

2.4.1 MX-1 Mixer

The Model MX-1 mixer is used when the DuoFlow system is **not** equipped with the Maximizer. The mixer may be used with or without a mixer barrel: the mixer body, mixer top, and mixer barrel are provided with the system; an optional mixer barrel extender is available. The mixer barrels fit between the mixer body and the mixer top and are used for higher flow rates. Refer to the following table to determine the appropriate mixer volume.

Table 2-9.
Mixer Barrels and Mixer Capacity for the MX-1 Mixer

Flow Rate	Barrel Extension	Capacity	Assembly Screws
less than 1ml/min	none	263 μ l	10-32 x 5/8" (1.6 cm)
1 to 10 ml/min	750 μ l Barrel Extender	750 μ l	10-32 x 7/8" (2.2 cm)
10 to 40 ml/min	2.0 ml Barrel Extender	2.0 ml	10-32 x 1-1/2" (3.8 cm)

The procedure for changing the mixer capacity is provided at the end of this section.

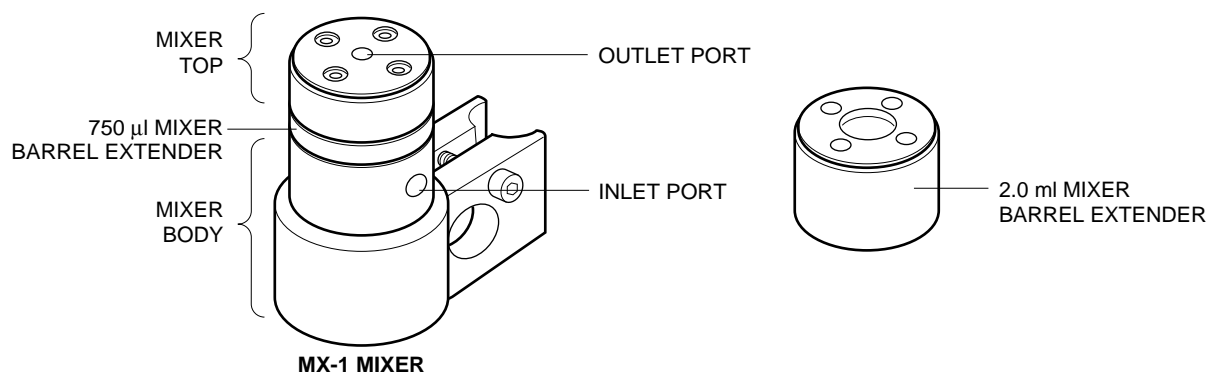


Figure 2-3. MX-1 Mixer and Mixer Barrel Extender

2.4.2 Maximizer Mixer

The Maximizer mixer provides the larger capacity required when the Maximizer is used. The Maximizer mixer may be used with or without a mixer barrel: the mixer body, mixer top, and mixer barrel are provided with the system; an optional mixer barrel extender is available. The mixer barrels fit between the mixer body and the mixer top and are used for higher flow rates. Refer to the following table to determine the appropriate mixer volume.

Table 2-10.
Mixer Barrels and Mixer Capacity for the Maximizer Mixer

Flow Rate	Barrel Extension	Capacity	Assembly Screws
0.5 to 10 ml/min	none	750 μ l	1/4-20 x 1/2" (1.3 cm)
10 to 40 ml/min	5 ml Barrel Extender	5 ml	1/4-20 x 1 1/2" (3.8 cm)
40 to 80 ml/min	12 ml Barrel Extender	12 ml	1/4-20 x 3 1/4" (8.2 cm)

The procedure for changing the mixer capacity is provided in the following section.

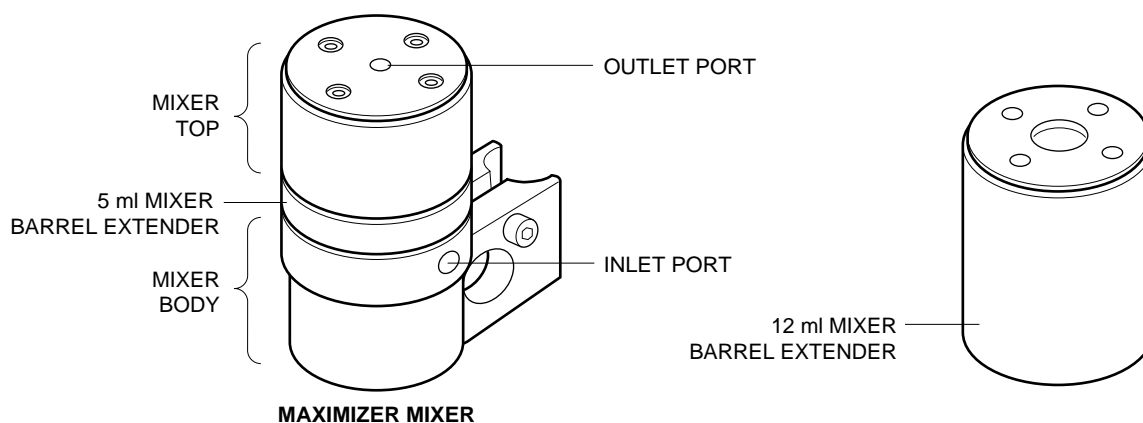


Figure 2-4. Maximizer Mixer and Mixer Barrel Extender

2.4.3 Changing Mixer Capacity

Be sure to follow these directions carefully. The mixer may leak if it is assembled incorrectly, or if an O-ring is not correctly placed in the O-ring groove.

Note: Flush any hazardous material from the system. Drain fluid from the mixer and disconnect the mixer plumbing and cables.

1. If you will be changing the mixer capacity, use the information in Table 2-9 and Table 2-10 to select the appropriate screws.
2. Use the hex key provided to remove the four screws from the top of the mixer (see Figure 2-5).
3. Remove the mixer top and turn it upside down to remove the O-ring. If the O-ring does not easily dislodge, use your fingers to remove it.
4. Refer to Figure 2-5 for re-assembling the mixer. Make sure that the magnetic stir bar lies flat. If the standard mixer barrel is to be used, assure the O-ring groove faces up.
5. Place an O-ring in each O-ring groove. If you are using only the mixer body, only one O-ring is required. If the mixer barrel is used, two O-rings are required.

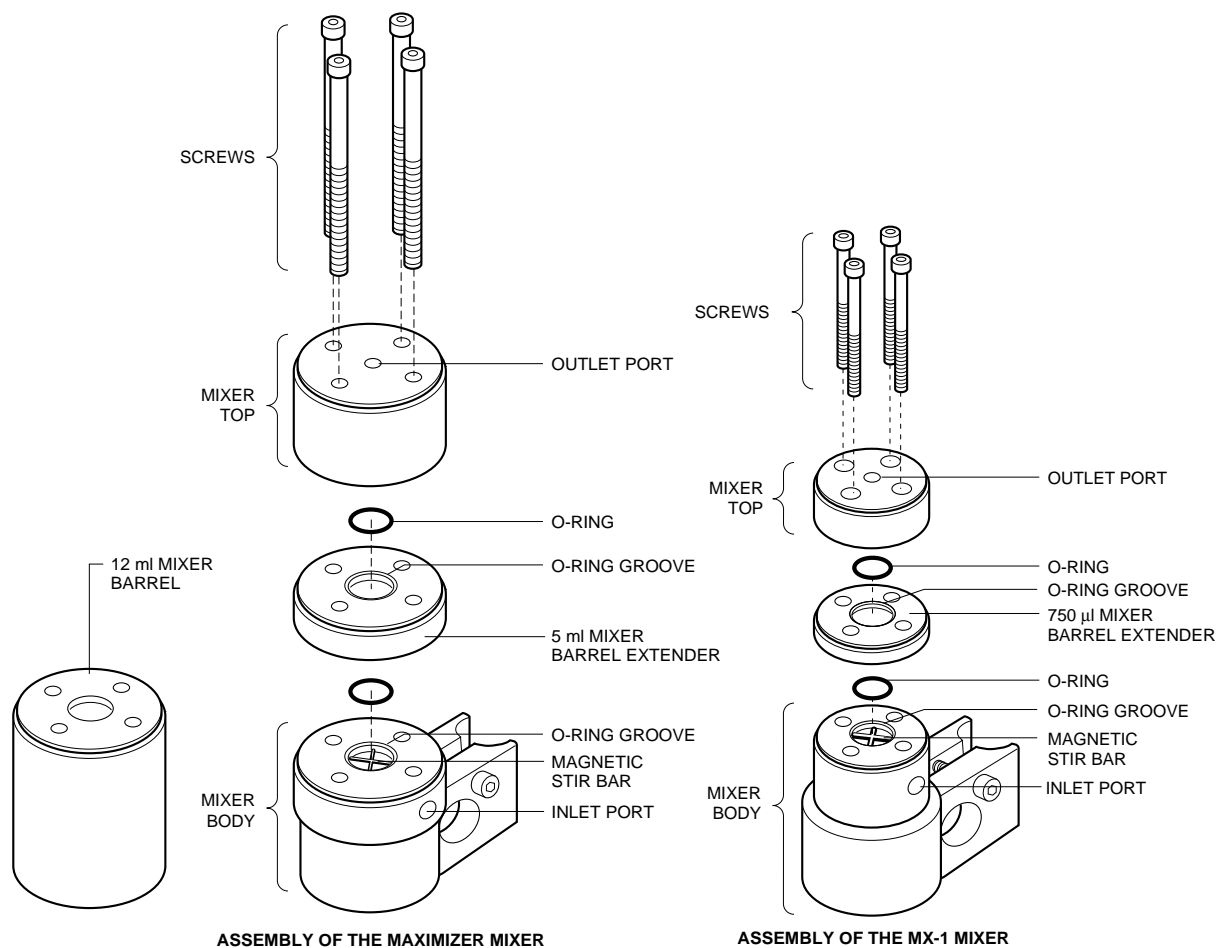


Figure 2-5. Assembly of Mixers

2.5 DETECTION SYSTEMS

The DuoFlow system supports the following detection and monitoring devices:

- UV Detector
- Conductivity Monitor
- QuadTec UV/VIS Detector

2.5.1 UV Detector

The UV detector is a single beam, fixed wavelength UV absorbance detector specifically designed for high resolution protein chromatography.

- Available in several configurations.
- Rack mountable and portable, which enables it to be positioned close to a column outlet for better resolution and decreased peak band broadening.
- Optional 214 nm filter and zinc lamp available for sensitive peptide analysis.

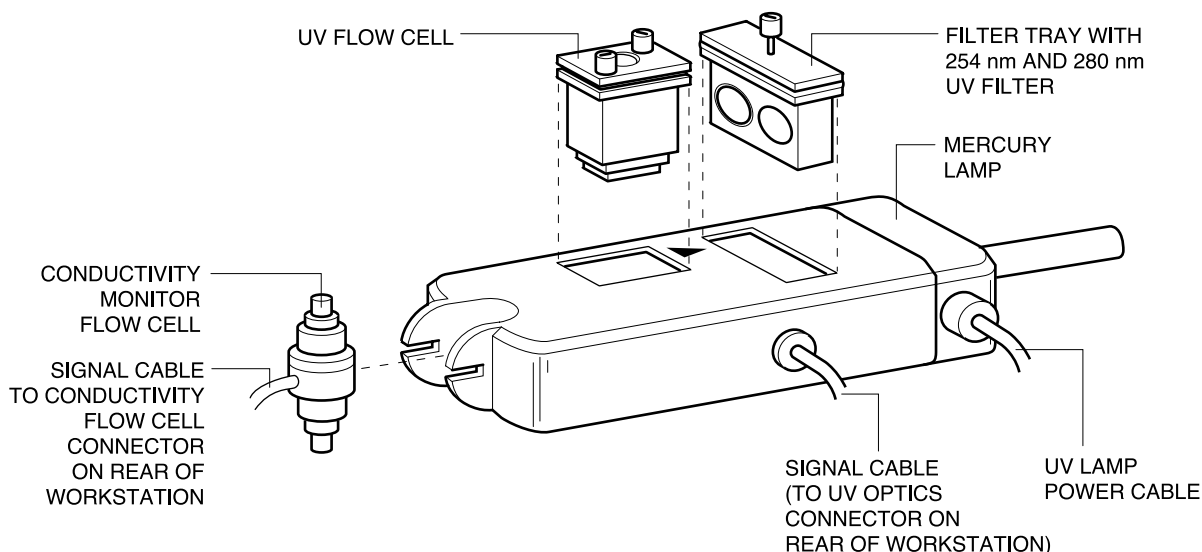


Figure 2-6. UV Detector, with Mercury Lamp, 254 & 280 nm Filters, and Conductivity Flow Cell

The UV detector consists of the optics bench, a filter tray, a flow cell, and a lamp. It is designed to hold the Conductivity monitor flow cell. The following configurations are available:

- **Mercury Lamp and Filters.** The mercury lamp comes installed in the optics module along with 280 nm and 254 nm filters. The filters are both held by a single tray. To switch filters, rotate the filter holder. Filters for different wavelengths are also available from Bio-Rad. These include 365 nm, 405 nm, 436 nm, and 546 nm filters.
- **Zinc Lamp and 214 nm Filter.** The zinc lamp and 214 nm filter are optional. The zinc lamp attaches directly to the optics module in place of the mercury lamp. (Refer to Figure 2-7)

Two flow cells are available with the detector. Both use 1/4-28 flat-bottom fittings.

- Analytical 5 mm flow cell. This flow cell is recommended for high resolution detection. This flow cell has a 5 mm path length, a volume of 16 μ l, and is rated to 750 psi at flow rates between 0.1 and 10 ml/min.
To reduce the risk of an entrapped air bubble causing an unstable baseline, the 5 mm flow cell should be used with the backpressure regulator (see page 2-52). Connect the backpressure regulator after the Conductivity monitor in the system plumbing.
- Preparative 2 mm flow cell. This flow cell is recommended for most applications which demand less sensitivity, for flow rates greater than 10 ml/min, or when working with high protein concentrations. It has a 2 mm path length, a volume of 30 μ l, and is rated to 750 psi.

The UV detector receives power via the Workstation, to which it is connected by the UV lamp cable. The UV detector communicates with the system via the UV optics cable, which plugs into the UV optics connector on the back of the Workstation.

The UV detector sensitivity ranges from 0 to 2.0 AUFS (Absorbance Units Full Scale). The UV sensitivity range for the chart recorder is set in the system software in either the Manual or Run screens. A Zero Baseline button is available in the Manual and Run screens and a programmable Zero Baseline command is available as part of a method protocol. Refer to the discussion of the chart recorder for setting the chart recorder range, page 2-55.

To replace an expired lamp, refer to Chapter 11, Maintenance, or to the instruction sheet for the replacement lamp.

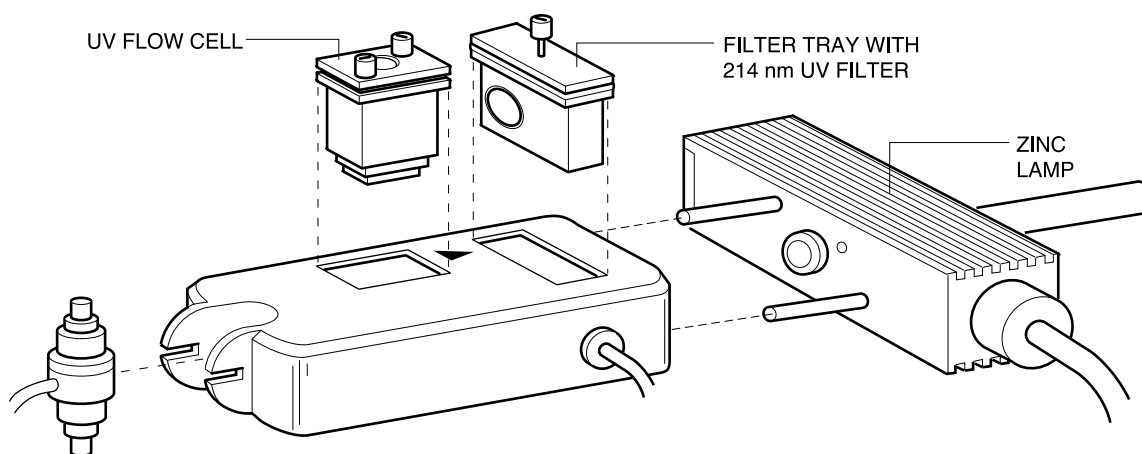


Figure 2-7. UV Detector, with Zinc Lamp, 214 nm Filter, and Conductivity Flow Cell

To change the UV flow cell or UV filter:

If the system has been used, make sure that any hazardous material has been flushed from the system and the pumps are not running. Drain fluid from the UV detector and disconnect its plumbing and cables.

1. To remove the UV flow cell, loosen the flow cell thumbscrews and lift the flow cell out.
2. To insert a flow cell, place it into the UV detector and tighten the thumbscrews.
3. To change the UV filter, loosen the flow cell thumbscrews and lift the filter tray out.
4. Rotate the filter tray to use the correct filter, and then place it into the UV detector and tighten the thumbscrews.

2.5.2 Conductivity Monitor

The Conductivity monitor, included with all DuoFlow systems, measures fluid conductivity to track the accuracy of a salt gradient. This data is useful for optimizing purification protocols and column cleaning procedures. Conductivity monitor sensitivity ranges from 0 to 500 mS/cm. The Conductivity monitor consists of the following:

- Flow cell with Inlet/Outlet ports: To connect the tubing, use 1/4-28 flat bottom fittings. The flow cell allows flow in either direction. The flow cell can be plumbed immediately after the UV flow cell or at any other point in the flowpath. It is designed to fit into a receptacle on the UV detector (see Figure 2-7). The volume in the cell is a nominal 6 μ l.
- Signal cable: To connect to the back of the Workstation. Electrical power for the Conductivity monitor is drawn from the signal cable. If a Maximizer is in use, connect to its COND port.

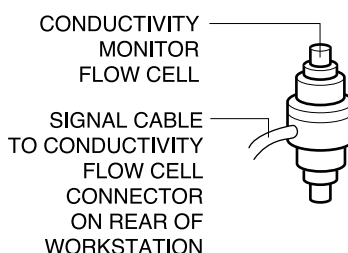


Figure 2-8. Conductivity Monitor

The Conductivity monitor can be calibrated by either entering the cell constant or by calibrating the cell against a known standard. The **Conductivity Flow Cell Constant Calibration** feature is found under the Utilities drop-down menu. The cell constant can be found on the tag attached to the cable.

The Conductivity sensitivity range for the chart recorder is set in the system software Manual and Run screens. When setting the range, choose one that will accommodate the maximum conductivity expected during the chromatography run. Keep in mind that although the chart recorder range can be manipulated during a chromatography run, the data will be stored unattenuated, allowing for re-scaling at a later time.

2.5.3 QuadTec UV/VIS Detector

The BioLogic QuadTec UV/Vis detector enables four wavelengths to be monitored simultaneously and displayed on the DuoFlow Controller. The QuadTec detector has a wavelength range of 190-370 nm using the deuterium lamp and 370-740 nm with the halogen lamp. The deuterium lamp is standard and is required for the detection of peptides, proteins, and nucleic acids. Both lamps are pre-aligned, calibrated, and user-serviceable.

Wavelengths are selected through the use of a moveable grating monochromator with an accuracy of ± 1 nm in 1 nm steps. When using the deuterium lamp for the monitoring of wavelengths >380 nm, an automatic cutoff filter is activated. Because very little light of wavelengths greater than 400 nm is emitted from a deuterium lamp, the halogen lamp is required for routine detection above 380 nm.

The QuadTec includes the following:

- 3mm Peek Flow Cell
- 10-32 Fingertight fittings (quantity 4)

- System Cable 25, RS232. To connect to the Instrument Control Module (ICM). If a Maximizer is in use, connect to its COM1 port instead of the ICM.
- Power cord

An optional 2mm PEEK flow cell is available for flow rates up to 80 ml/min including 10-32 long Fingertight fittings (quantity 4).

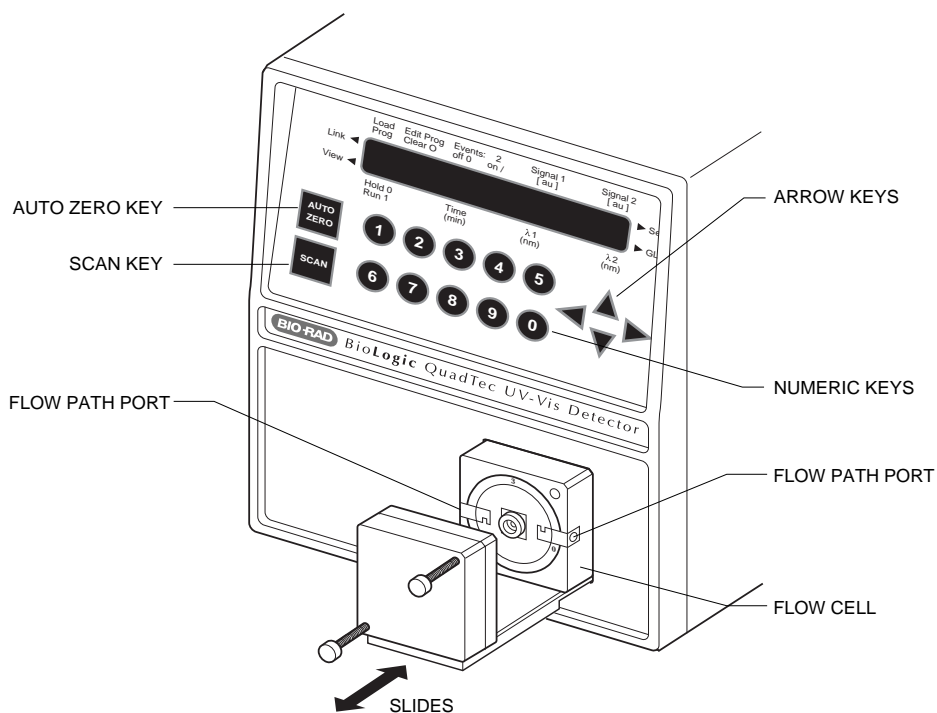


Figure 2-9. QuadTec UV/Vis Detector

For a complete discussion of the QuadTec detector, refer to its separate documentation.

The QuadTec detector is shipped with a “dummy” flow cell installed. Before operating the detector install the biocompatible 3mm PEEK flow cell. Refer to Figure 2-9 and the procedure below. Before removing the dummy cell refer to section 5.1 in the QuadTec instruction manual for instructions to record signal and reference values.

1. Make sure the detector power is OFF.
2. Loosen the two knurled screws on the front of the flow cell and gently pull them out. This allows the flow cell housing to slide out.
3. Remove the dummy cell by gently pulling it upward. Save the dummy cell in a secure place. It is required for measuring signal and reference output values for the lamps. See Section 5.1, Checking the Status of D2 Lamp, in the QuadTec instruction manual for instruction on recording signal and reference values.
4. Insert the new flow cell and make sure that the engraved specifications point towards the user and that the fixing hole on the back side of the cell meets the corresponding metal pin of the detector's housing.
5. Slide the complete system towards the detector, insert the two knurled screws, and tighten them by hand.
6. Attach the 10-32 Fingertight fittings and tubing to the inlet and outlet ports and use a syringe to rinse the flow cell with 10-20 ml of 100% analytical grade methanol, followed by 5 ml distilled deionized (DDI) water.

2.5.4 pH Monitor

The BioLogic DuoFlow pH monitor enables direct monitoring of pH conditions during a run. The pH monitor is optional for all DuoFlow systems.

The pH monitor consists of the following:

- **Flow cell:** The PEEK flow cell has a swept volume of approximately 80 μl when the pH electrode is inserted. The flow cell mounts to the BioLogic rack using an attached mounting bracket, which can also be used to attach the UV detector and Conductivity monitor. The flow cell should be positioned so that the flow path is angled upward to promote bubble clearance. Plumb the flow cell with the inlet tube attached to the lower port and the outlet tube attached to the upper port. The inlet and outlet ports are threaded for use with 1/16" (1.6 mm) OD tubing and 1/4-28 flat bottom fittings. The pH monitor is designed for flow rates up to 80 ml/min and flow cell pressure less than 75 psi, and therefore should be plumbed downstream of the backpressure regulator.
- **pH electrode:** The pH electrode is sealed Calomel consisting of a pH and a reference electrode built into the same body. The sealed reference design eliminates the need to add electrolyte solutions and minimizes reference dryout. The Calomel electrode is fully compatible with buffers (such as Tris) that may not be compatible with the Ag/AgCl (silver/silver chloride) containing electrodes.
- **Signal Cable:** To connect to the Signal Import Module (SIM. Refer to page 3-12 for connecting the SIM to the pH monitor.) If a Maximizer is in use, connect to its pH BNC port.

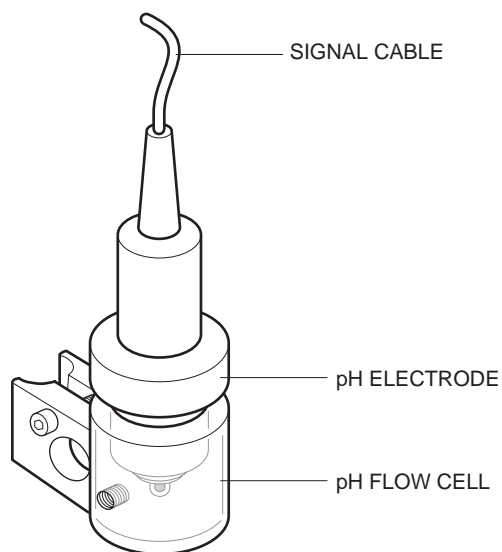


Figure 2-10. pH Monitor

The pH electrode and flow cell are described in detail in their separate documentation.

2.5.5 Other Detectors

Detectors other than those offered by Bio-Rad may be used when connected to the Signal Import Module (SIM.) Refer to page 2-53.

2.6 VALVES

BioRad offers an extensive variety of valves enabling greater flexibility for multiple sampling and application options. DuoFlow systems control 3 AVR high pressure valves and 3 SV low pressure valves. DuoFlow systems with a Maximizer double the number of valves to 6 AVR high pressure and 6 SV low pressure valves. Methods that include automated injection, buffer selection, column switching, and large volume fraction collection are easily performed using various valve configurations.

For further discussion of valving applications and protocols, refer to Chapters 8-10.

2.6.1 AVR7-3 Sample Inject Valve

The AVR7-3 sample inject valve is a 7-port, 3-position valve for injecting samples and is an essential component of all DuoFlow systems. It is rated to 3500 psi (233 bar) and is designed with non-metallic wetted parts and minimal internal dead volume. Some features of this valve include:

- Patented make-before-break design (MBB™) prevents pressure spikes when the valve rotates from one port to another, eliminating baseline interferences and prevents pump shutdowns due to transient over pressure situations. This is especially beneficial when using more fragile low pressure columns or flow sensitive detectors (e.g. refractive index).
- Applications include sample injection, reverse flow chromatography and two-column switching.
- A valve rebuild kit is available.

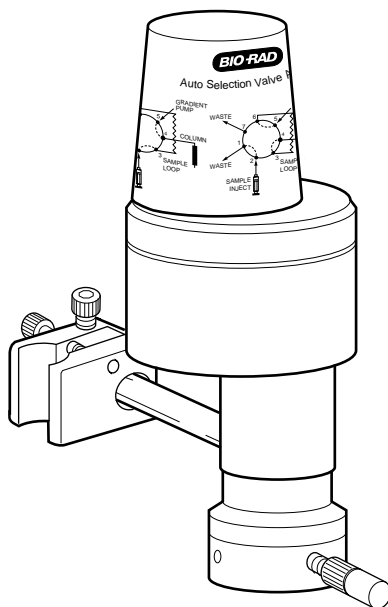


Figure 2-11. AVR7-3 Sample Inject Valve

The three valve positions are Load (position 1), Inject (position 2), and Purge (position 3). See Figure 2-12. Load is the default position when the system is powered up or at the end of a method run unless configured differently from the Edit User Preferences window, available from the Options menu in the system software.

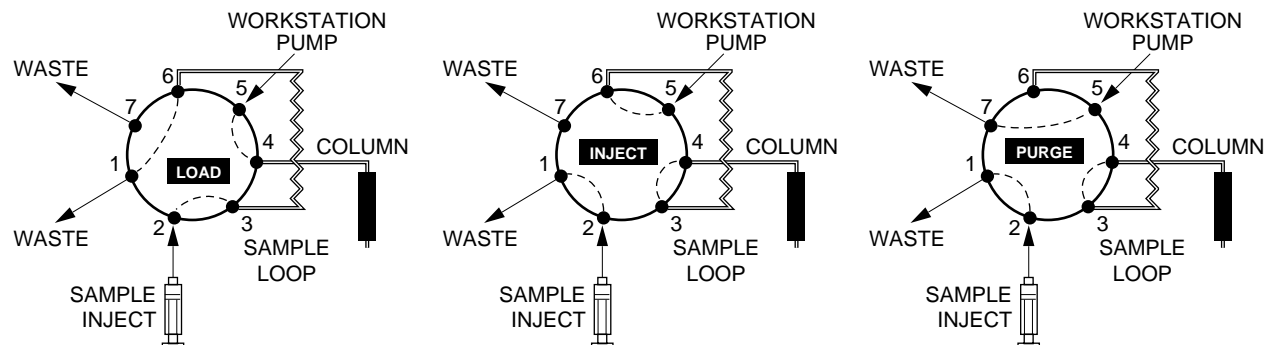


Figure 2-12. Sample Load Positions

The valve uses 1/16" (1.6 mm) OD tubing and 1/4-28 fittings. Sample loop sizes are available from 50 μ l loop (in the Starter kit included with all systems) to a maximum of 5 ml PEEK loop. Larger volume injections may be obtained using Dynaloops, additional valves and/or an auxiliary pump.

Connect the AVR7-3 valve signal cable to any of the available Automated Valve connectors on the back of the Workstation (ports 4, 5, or 6). If a Maximizer is in use connect to ports 7, 8, or 9 before those on the Workstation. If more than 3 valves are desired, you may connect additional valves to ports 4, 5, and 6 on the Workstation. All valves will be active.

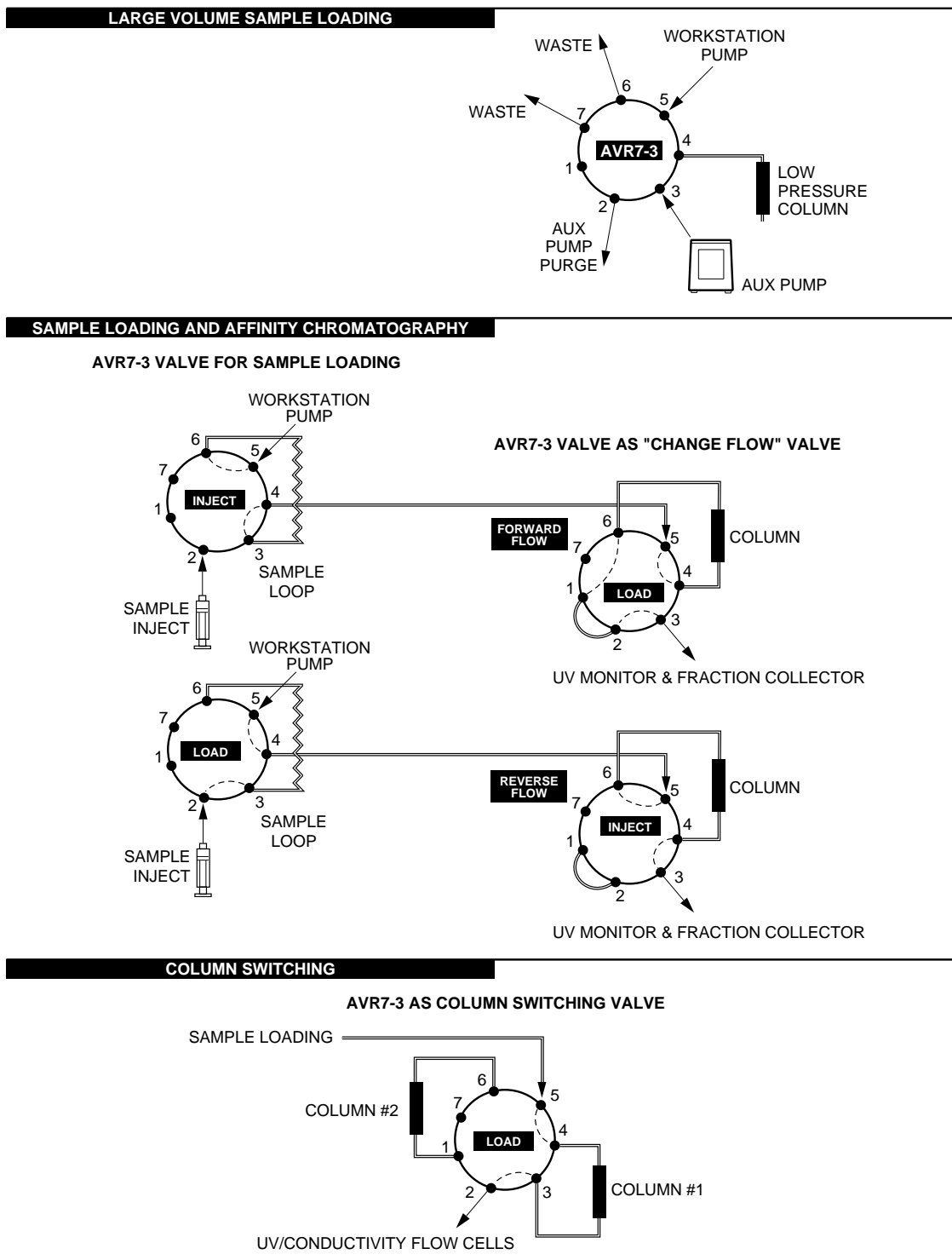


Figure 2-13. Examples of AVR7-3 Valve Tubing

2.6.2 AVR9-8 Stream Select Valve

The AVR9-8 stream select valve is an 9-port 8-position valve. This valve is optional for all DuoFlow systems. The valve is rated at 3500 psi (233 bar) and is designed with non-metallic wetted parts for bio-compatibility and minimal internal dead volumes.

- The AVR9-8 is ideal for stream selection, column switching, and large volume fraction collection.
- The AVR9-8 stream select valve uses a patented make-before-break design (MBB) that prevents pressure spikes when the valve rotates from one port to another, eliminating baseline interferences, and prevents pump shutdowns due to transient over pressure situations. This is especially beneficial when using more fragile low pressure columns or flow sensitive detectors (e.g. refractive index).
- A valve rebuild kit is available.

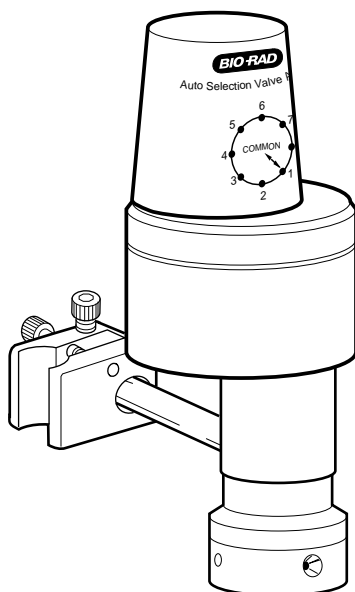


Figure 2-14. AVR9-8 Stream Select Valve

The valve's use dictates its plumbing. For example:

- When two AVR9-8 valves are used, up to eight columns may be run sequentially.
- An AVR9-8 expands the number of buffers or solvents in use.
- When used in conjunction with an auxiliary peristaltic pump (such as the Bio-Rad Model EP-1 Econo pump or EGP Econo Gradient Pump) for loop filling and an AVR7-3 inject valve, the AVR9-8 valve may be used to select up to eight samples for consecutive chromatography runs.

Position 1 on the AVR9-8 is the default position when the system is powered up or at the end of a method run unless configured differently from the Edit User Preferences window, available from the Options menu.

The valve uses 1/4-28 fittings and 1/16" (1.6 mm) OD PEEK tubing supplied with the fitting kit when plumbing these valves.

Connect the AVR9-8 valve signal cable to any of the available automated valve connectors on the back of the Workstation (ports 4, 5, or 6). If a Maximizer is in use connect to ports 7, 8, or 9 before those on the Workstation. If more than three valves are desired, you may connect additional valves to ports 4, 5, and 6 on the Workstation. All valves will be active.

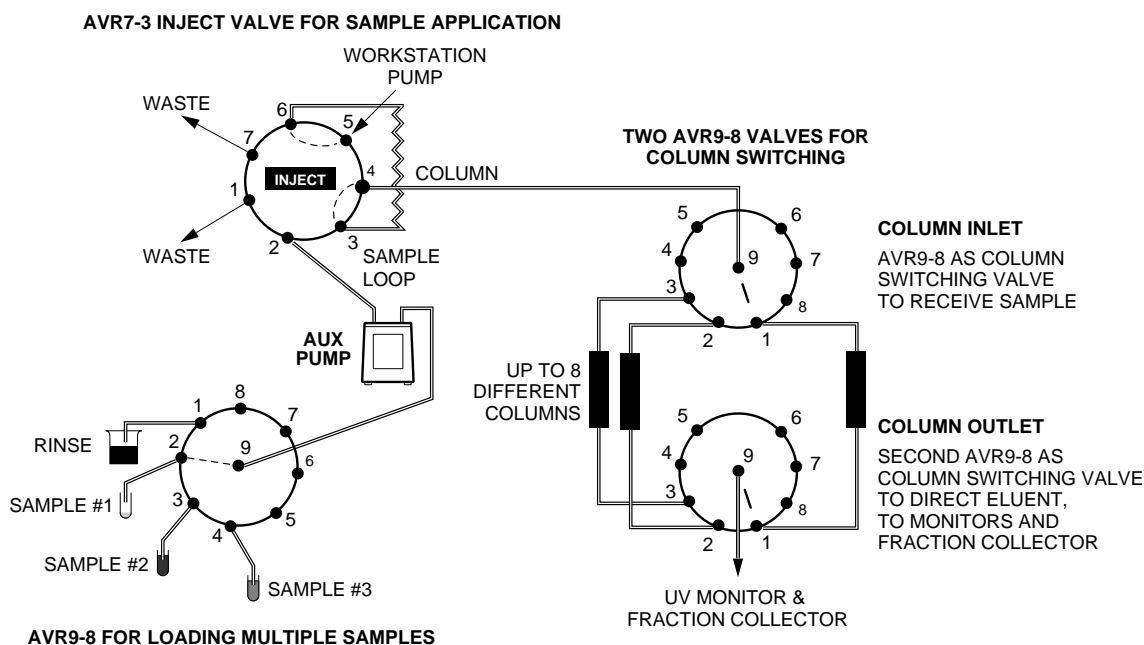


Figure 2-15. Two Examples Using AVR9-8 Valves

2.6.3 SV5-4 Buffer Select and Automated Sample Loading Valve

The SV5-4 valve is a low pressure, 5-port, 4-position valve used for automatic buffer selection and sample loading. The SV5-4 valve may be used for:

- Buffer and/or sample selection when placed before the Workstation pumps or an auxiliary load pump. An SV5-4 valve connected to a Workstation pump inlet enables access to four separate solutions.
- Fraction collection when placed after the column.
- Purging or rinsing all tubing lines for cleaning purposes.

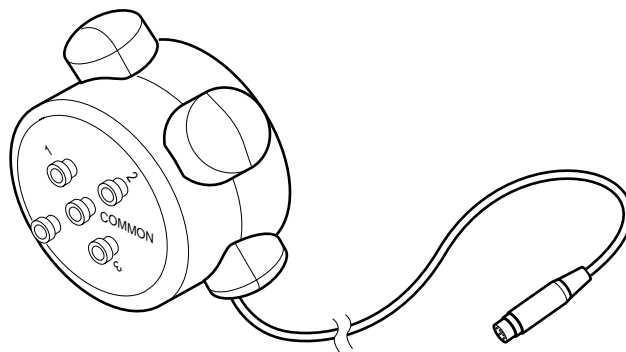


Figure 2-16. SV5-4 Buffer Select Valve

Use 1/8" (3.2 mm) OD Teflon tubing and 1/4-28 fittings when plumbing the SV5-4 valve to the Workstation pump inlet. To prevent air from entering the system, unused ports should be closed with 1/4-28 plugs.

When loading samples directly through the Workstation pump, first filter the sample through a 0.45 μ m filter. Flush the valve pump and lines with a sanitizing solution to remove protein residues after the sample has been loaded; protein residues might otherwise reduce pump piston seal lifetime.

Connect the SV5-4 valve cable to any of the available solenoid valve connectors on the back of the Workstation (ports 1, 2, or 3). If a Maximizer is in use, connect to ports 4, 5, or 6 before those on the Workstation.

From the Manual screen you may manually operate each inlet port on the SV5-4. Manual valve control is useful for the following:

- When priming the tubing and the valves with buffer and or sample prior to starting a method.
- For purging all tubing lines for cleaning purposes.

Prior to programming a method in the Protocol screen, you may name each of the valves positions in the Setup screen. The name that you apply in the Setup screen will appear in the method Protocol screen when you program an Isocratic Flow, Change Valve, or Linear Gradient step.

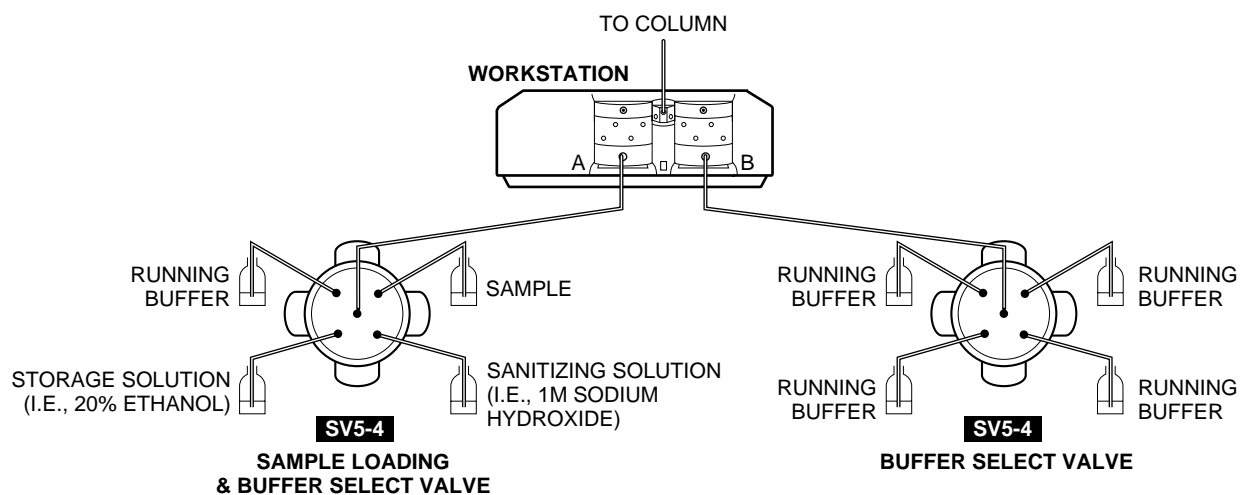
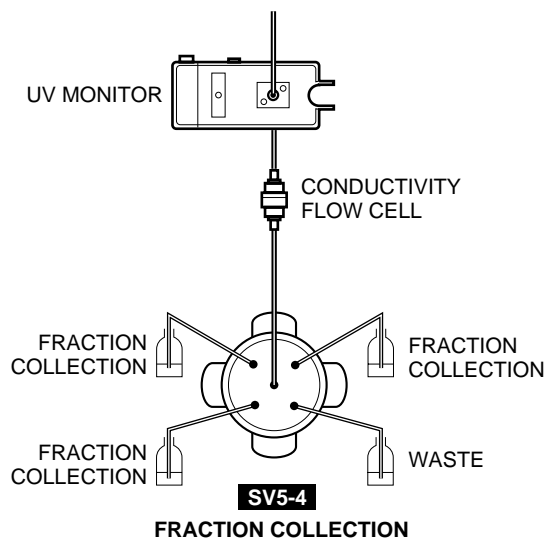
SV5-4 FOR BUFFER AND SAMPLE LOADING**SV5-4 AS FRACTION COLLECTOR**

Figure 2-17. Two Examples using SV5-4 Valves

2.6.4 SVT3-2 Diverter Valve

The SVT3-2 diverter valve is a low pressure 3-port, 2-position valve. This valve may be used in a number of ways:

- To divert the eluant stream from the detectors to a fraction collector or a waste container.
- As a sample select valve when placed before Workstation pump A. The Maximizer may not be used in this application.
- As a water rinse valve when placed before Workstation pump B.

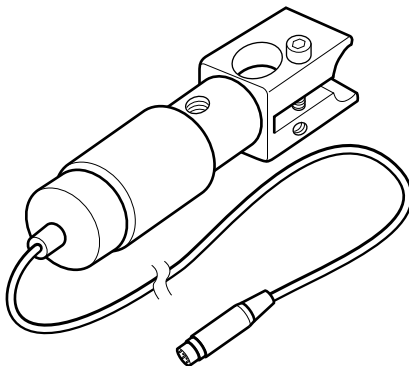


Figure 2-18. SVT3-2 Diverter Valve

Connect the SVT3-2 valve's signal cable to any of the available solenoid valve connectors on the back of the Workstation (ports 1, 2 or 3).

Plumbing connections depend on the valve's use:

- Plumbing as a diverter valve: Use 1/16" (1.6 mm) OD PEEK tubing and 1/4-28 fittings. Use orange PEEK 0.020" (0.51 mm) ID tubing for the F10 pumps, and green PEEK 0.030" (0.76 mm) ID tubing for the F40 pumps.
- Plumbing before a pump: Use 1/8" (3.2 mm) OD tubing and 1/4-28 fittings.

From the Manual screen you can manually switch inlet ports on the SVT3-2. Manual valve control is useful for the following:

- When priming the tubing and the valves with buffer prior to starting a method.
- For purging or rinsing all tubing lines during cleaning process.

In the Setup screen you can name each of the two valve positions. For example, when the SVT3-2 is placed before the Workstation pump, you can identify the valve by the name or composition of the buffer. The name that you apply in the Setup screen will appear in the method Protocol screen when you program an Isocratic Flow step, a Change Valve step, or a Linear Gradient step.

When used as a diverter valve with a Model 2110 or generic fraction collector, this valve should be named as such in the Setup screen. Position 1 of this valve is Waste; position 2, Collect.

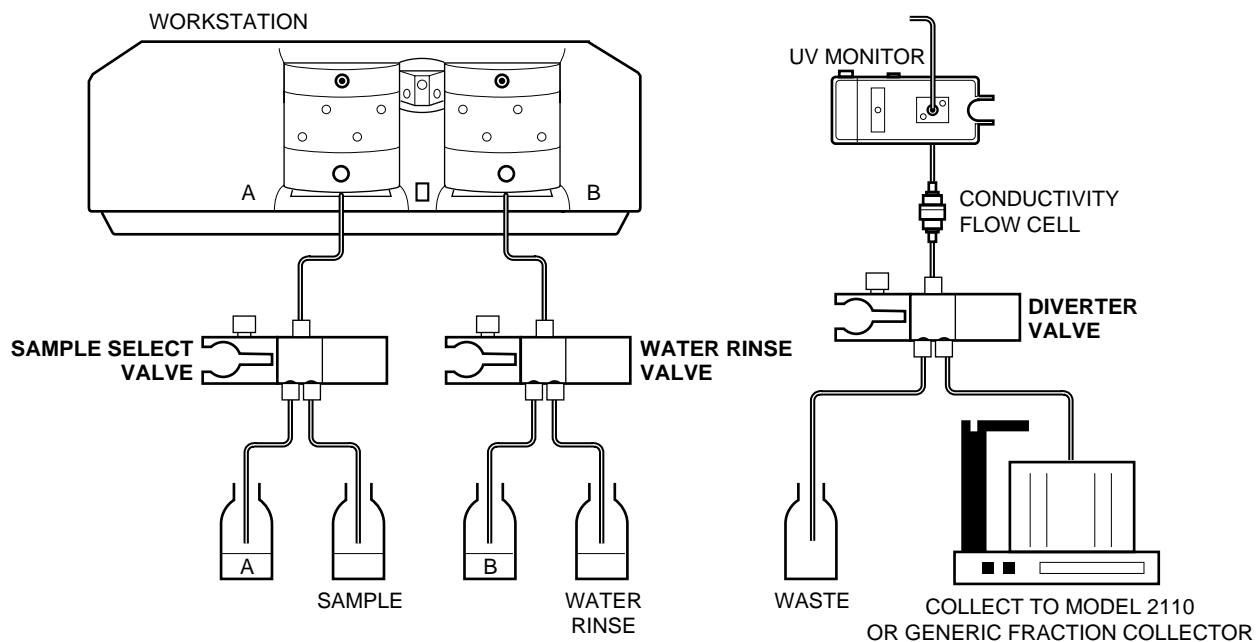


Figure 2-19. Two Configurations of the SVT3-2 Valve

When used as a diverter valve with a Model 2110 or generic fraction collector, this valve should be named as such from the Setup screen. Position 1 of this valve is Waste; position 2, Collect.

2.7 FRACTION COLLECTORS

The DuoFlow features several options for fraction collection:

- BioFrac fraction collector
- Model 2110 fraction collector
- Model 2128 fraction collector
- Non-Bio-rad fraction collectors

The BioFrac fraction collector, the Model 2110 fraction collector, and the Model 2128 fraction collector are programmable and can be run via BioLogic software version 4.0 or higher.

2.7.1 BioFrac Fraction Collector

The BioFrac functions with the BioLogic DuoFlow system and as a stand-alone fraction collector with the BioLogic HR and non Bio-Rad chromatography systems. It is suited for both analytical and preparative applications.

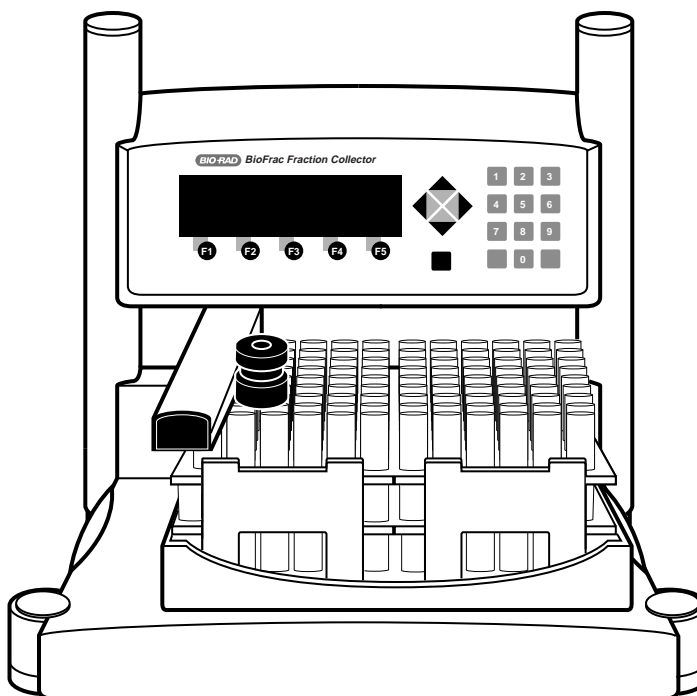


Figure 2-20. BioFrac Fraction Collector

Features of the BioFrac include:

- It can be used with any chromatography system at flow rates up to 100 ml/min.
- It can collect in time or drop mode. It can also collect in volume mode when connected to the Model EP-1 Econo pump.
- A diverter valve mounted on either side of the vertical bars can divert unwanted eluant to waste, and it diverts flow between fraction changes.

The BioFrac moves in a serpentine X,Y motion over each collection vessel dispensing fluid while programmable column or row movement may be selected when using microplates or microtiter tubes. Fourteen collection choices are possible with a total of nine racks. The BioFrac accommodates 12-20 mm tube diameters, microtubes, and scintillation vials in a variety of diameters. The optional ice bath/microplate rack allows collection into 13 mm diameter tubes, microplates, and microtiter tubes that meet SBS standards required for automated microplate systems. A manually adjustable dispenser arm allows the BioFrac to collect into test tubes with heights up to 150 mm.

The following racks are available.

Table 2-11.
BioFrac Racks Available

Rack Name	Description	Capacity (Format)
F1	12-13 mm diameter tube; 150 mm height	2 racks (6x15), 180 tubes
F2	15-16 mm diameter tube; 150 mm height	2 racks (5x12), 120 tubes
F3	18-20 mm diameter tube; 150 mm height	2 racks (4x10), 80 tubes
H1	1.5-2.0 ml capless microtubes	4 racks (6x7), 168 tubes
H2	0.5 ml capless microtubes	4 racks (7x9), 120 tubes
H3	16 mm scintillation vials	4 racks (5x6), 120 tubes
H4	30 mm scintillation vials	4 racks (2x3), 24 tubes
Ice Bath, Microplate rack	Ice bath for 13 mm diameter tubes, standard microplate or microtiter tubes (SBS standard format)	1 rack (10x12), 120 tubes 4 plates (12x8), 96 wells 4 plates (8x6), 48 wells 4 plates (4x6), 24 wells 4 plates (3x4) 12 wells
Prep-20	Preparative rack	bottle size fractions (20 funnels)

The BioFrac communicates with the DuoFlow Controller via the Instrument Bus. To connect the BioFrac to the bitbus use either of the instrument bus connectors on the rear of the fraction collector. The BioFrac fraction collector supports collection schemes such as Collect All, Threshold, Collection Windows, and Threshold + Collection Windows (all with Delay volume, if required). The BioFrac diverter valve minimizes liquid spills during fraction advances and can be mounted on either side of the fraction collector's upright supports.

The BioFrac is plumbed to the DuoFlow system using 0.020" diameter PEEK tubing and 1/4-28 fittings for flow rates up to 20 ml/min or with 0.030" diameter PEEK tubing and 1/4-28 fittings for flow rates up to 100 ml/min.

This instrument is described in detail in its separate documentation.

2.7.2 Model 2110 Fraction Collector

The Model 2110 is programmed in the DuoFlow software and can function as a stand-alone with non Bio-Rad systems. It uses a stationary, drop-dispensing head and collects up to 80 fractions in a motor-driven carousel. It also uses standard 13 x 100 test tubes. An optional adapter is available for use with 1.5 ml microcentrifuge test tubes. The Model 2110 includes the following features:

- In stand-alone mode the Model 2110 accepts small chromatography columns that can mount directly to the drop-forming arm to minimize dead volume.
- It can collect from 1 drop (50 μ l) to 9 ml fraction in the 13 x 100 test tubes or 1.5 ml micro test tubes.
- It is coldroom compatible.

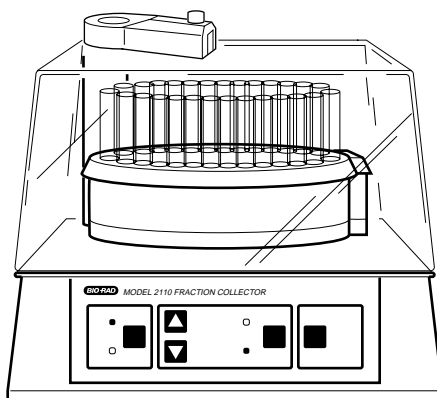


Figure 2-21. Model 2110 Fraction Collector with Optional Dust Cover

The Model 2110 is connected to the Maximizer or Workstation with system cable 5, bare wires to DB-9 via the AUX connector, black wire to pin 5 and white wire to pin 9. To prevent shorting, cut or tape wires not in use. If a Maximizer is being used, the Model 2110 should be connected to it rather than to the Workstation.

The Model 2110 is plumbed to the DuoFlow system using 1/16" (1.6 mm) OD inlet tubing connecting directly to the fraction collector's drop former, without the need for additional fittings.

The SVT3-2 diverter valve is required by the Model 2110 for collection schemes other than "Collect All." When this valve is configured as the *fraction collector diverter valve* in the Setup screen, additional collection parameters (Collect All, Threshold, Collection Windows, and Threshold + Collection Windows) become available in the Protocol screen.

When controlled by the DuoFlow system, fraction collection is specified by volume. The fraction collection scheme is programmed in the Protocol screen of the software where a Delay Volume feature is standard (see Chapter 7 for discussion of Delay Volume.)

Note: Collection by drop count is not available with the DuoFlow system.

This instrument is described in detail in its separate documentation.

2.7.3 Model 2128 Fraction Collector

The Model 2128 provides X/Y motion drop-dispensing across 5 available racks which accommodate a wide range of tube diameters and lengths, microtiter plates, microtubes, and “bottle size” fractions. It is suited for both analytical and preparative applications.

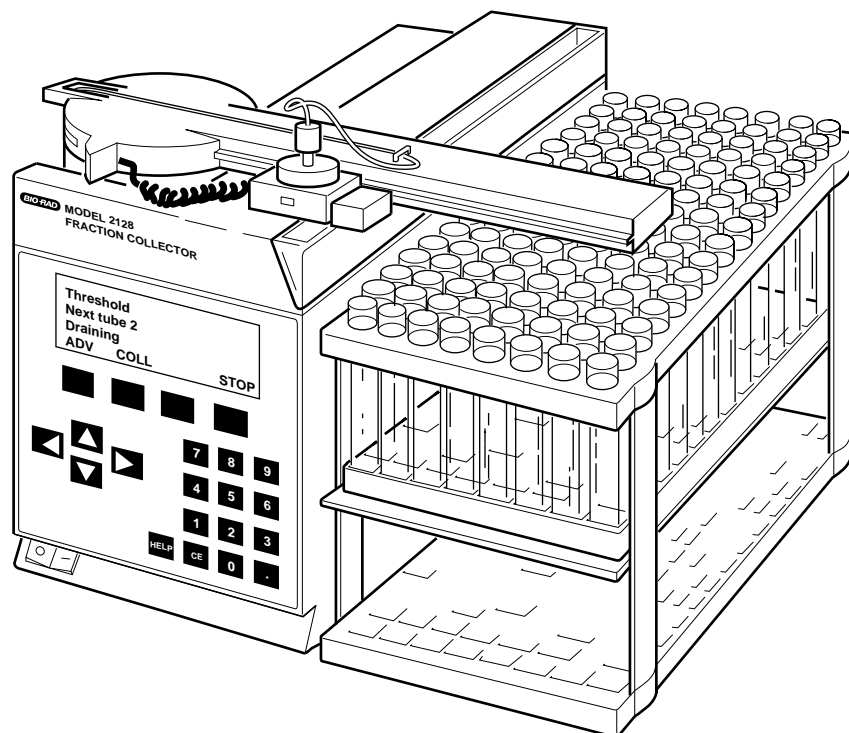


Figure 2-22. Model 2128 Fraction Collector

The following racks are available.

**Table 2-12.
Model 2128 Racks Available**

Rack Name (Software ID Number)	Tube Description	Capacity
Rack #1 (1)	12 and 13 mm diameter; 180 mm height (max.)	128 (16 X 8) tubes
Rack #2 (2)	16 to 18 mm diameter; 180 mm height (max.)	78 (13 X 6) tubes
Micro-Adapter (3)	Microplate (96 wells in 8 X 12 format)	3 plates (288 wells)
Micro-Adapter (4)	Microtubes (capless tubes required)	128 microtubes
Prep-Adapter (5)	Bottles of any size	10 bottles

The Model 2128 is controlled by the DuoFlow Controller via the Instrument Bus. Use the Instrument Bus connector on the rear of the fraction collector.

The Model 2128 is plumbed to the DuoFlow system using 1/16" (1.6 mm) OD inlet tubing and either of the following fittings:

- To connect directly to the dispenser head, use 1/4-28 flat bottom fittings.
- To connect to the optional on-arm diverter valve, use the 10-32 nut and ferrule fittings supplied with the valve.

When the Model 2128 is configured in the Setup screen, collection schemes such as Collect All, Threshold, Collection Windows, and Threshold + Collection Windows (all with Delay volume, if required) automatically become available in the Protocol screen. The starting and ending tube numbers also may be specified. The Model 2128's optional diverter valve mounts on the fraction collector's arm and minimizes liquid spills during fraction advances. If this valve is used, it is automatically sensed by the system and hence is **not** configured by the user in the Setup screen.

This instrument is described in detail in its separate documentation.

2.7.4 Generic Fraction Collectors

A non-Bio-Rad collector may be used as an integral part of the DuoFlow system providing its tube advance function can be initiated by an active low TTL signal (>100 ms). Electrical connection from the collector is to pin 5 (Frac Advance) and pin 9 (ground) of the Workstation's AUX connector. See the pin-out information in your collector manual.

With an optional SVT3-2 diverter valve configured as a *fraction collector diverter valve* in the Setup screen, the DuoFlow Controller will control all collection parameters including advanced features such as collection by Threshold and/or Collection Windows. If the optional valve is not used, then only Collect All is available.

Fraction advance marks to a Bio-Rad chart recorder are embedded on the UV signal from the Workstation.

2.8 SAMPLE LOADING OPTIONS

The BioLogic DuoFlow system supports several methods for sample loading. Typically a sample is loaded through a static fixed volume loop on the AVR7-3 inject valve. When large volume sample injections are required, the following options are available:

- Sample loading through the Workstation pumps: The SV5-4 or SV3-2 valve may be used to load large volumes of sample through the Workstation pumps. Refer to page 2-31 and page 2-33 for the discussion of these valves.
- Sample loading through a DynaLoop sliding-piston loop: When used with an AVR7-3 inject valve, the DynaLoop may be used for large volume sample loading or repetitive injections of smaller volumes of sample. The DynaLoop comes in two sizes: 25 and 90 ml volume.
- Sample loading through an auxiliary pump, such as the EP-1 Econo pump (EP-1) or the Econo Gradient Pump (EGP). When used with an AVR7-3 inject valve, an EP-1 or EGP pump may be used for large volume sample injections. (Placing an AVR9-8 valve before the pump inlet allows loading of multiple samples. Refer to the discussion of the AVR9-8 valve for details.)

2.8.1 DynaLoop Large Volume Sample Injection Loop

The DynaLoop, available in 25 ml and 90 ml sizes, is a sliding-piston dynamic loop for injection of a large sample volume or repetitive injections of smaller samples. The DynaLoops have a sliding piston that functions like a syringe. The loop is installed on the AVR7-3 in place of the static loop. (Refer to figure 2-24). Sample is loaded into the DynaLoop's sample end connector through port #2 on the AVR7-3 valve and may be loaded with a syringe or auxiliary pumps, for example Bio-Rad's Econo Gradient Pump or EP-1 pump. The loading of the sample pushes the DynaLoop's sliding seal towards the buffer end connector. As solution flows into the buffer end of the DynaLoop it pushes the sample into the system.

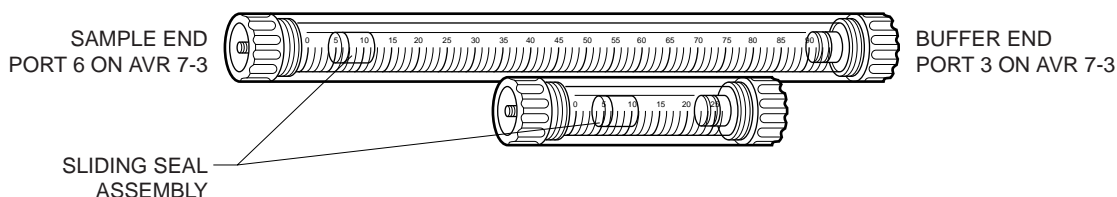


Figure 2-23. DynaLoop

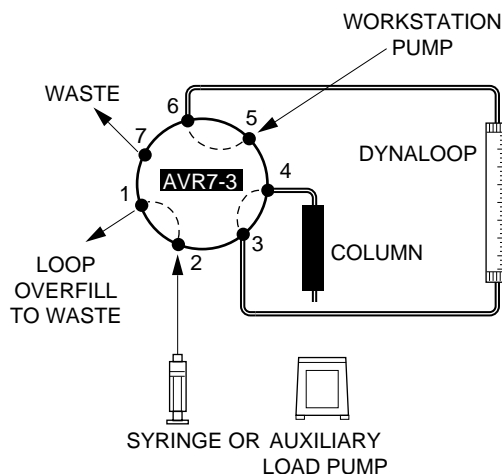


Figure 2-24. Plumbing the DynaLoop for use with an Inject Valve

When plumbed directly to the AVR7-3 inject valve, the DynaLoop functions just like a static sample loop. The sample end of the DynaLoop should be plumbed to port 3 and the buffer end to port 6 of the inject valve. The valve's operation and sample loading are controlled automatically, simplifying the sample injection process and insuring precise sample loading and gradient formation. The DynaLoop can be filled using a syringe or auxiliary pump.

Use a 1/4x28 to female Luer adapter between the sample load syringe and the AVR7-3 valve port 2 makes filling the loop much easier.

Consult the DynaLoop user's manual for additional information.

2.8.2 Model EP-1 Econo Pump

The Model EP-1 Econo pump is a two-channel, bi-directional, variable speed peristaltic pump for low-pressure chromatography. The EP-1 offers a full range of features to facilitate ease of use as a stand-alone pump or as an accessory to the DuoFlow System to load large volumes, especially onto low pressure columns. This instrument is described in detail in its separate documentation. Features of the EP-1 include:

- The EP-1 has preset calibration for common tubing sizes and manual calibration for nonstandard size tubing.
- It controls various fraction collectors, including the BioFrac, Model 2110, and Model 2128 fraction collectors.
- It can control the run length with a programmable total run volume.
- It is coldroom compatible.

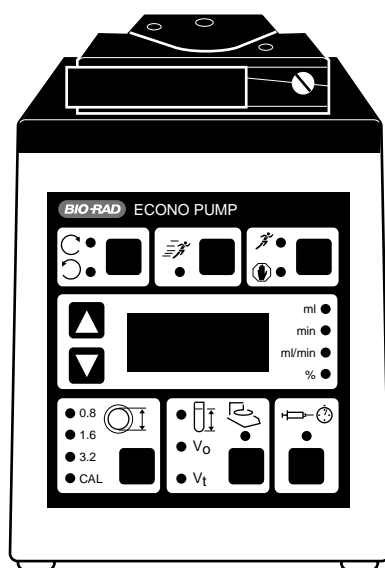


Figure 2-25. Model EP-1 Econo Pump

The EP-1 Econo pump is ideal for loading samples onto a low-pressure column. When using a low-pressure column, such as an Econo-Pac cartridge, remove the 40 psi backpressure regulator from the post-column position. Place it at the outlet of the Workstation pump. This will allow the check valves to properly seat and ensure pump flow performance.

The EP-1 Econo pump must contain firmware version 2.12 or higher to function with the BioLogic DuoFlow system. To confirm the firmware version, simultaneously press and hold down the Direction key and the down arrow key on the front panel of the EP-1 Econo pump. If the Econo pump does not have the correct firmware version, contact BioRad for information on how to upgrade the pump.

The EP-1 Econo pump receives start/stop signals only. The flow rate of the EP-1 Econo pump is set from the pump, and the flow rate is recorded in the yellow data entry boxes of the Load/Inject Sample dialog box.

The DuoFlow system starts/stops the Aux pump and cannot control the flow of the EP-1 Econo pump. The correct flow rate must be set at the EP-1 Econo pump.

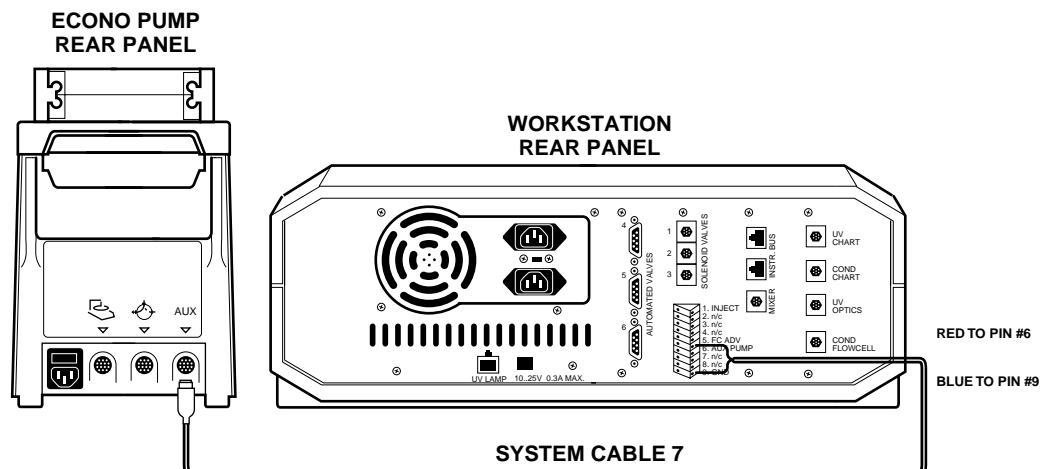


Figure 2-26. Connecting an EP-1 Econo Pump to the BioLogic DuoFlow Workstation

To connect the EP-1 Econo pump with the DuoFlow system, make the following cable connections:

1. On System Cable 7 locate the red and blue wires at the wire end of the cable and connect them to the DuoFlow Workstation.
 - a. Connect the red wire to pin 6 of the Aux connector.
 - b. Connect the blue wire to pin 9 of the Aux connector.
2. Plug the 8-pin mini-DIN connector into the Aux connector on the rear panel of the Econo pump.

PharMed tubing is recommended for use in the pumphead of the EP-1 Econo pump. For additional information on this pump, consult its user manual. The illustration below shows the Aux pump connected to port 3 of the Inject Valve.

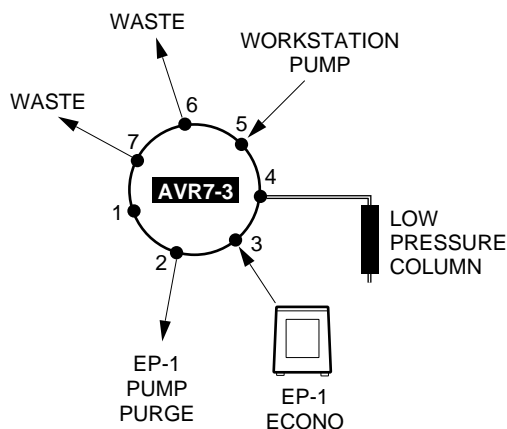


Figure 2-27. Example of Large Sample Loading using an Econo Pump

The EP-1 Econo pump can be used to load up to 7 samples sequentially when used with an AVR9-8 at the pump's inlet valve, as shown below.

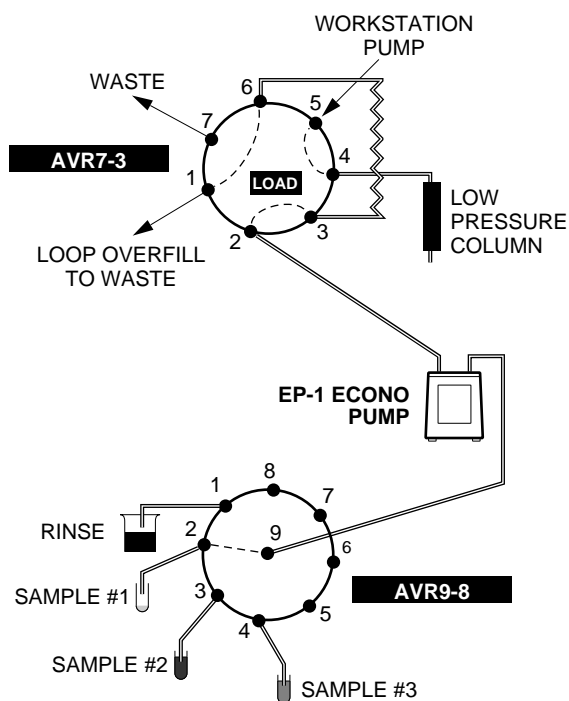


Figure 2-28. Example of Multiple Sample Loading using an Econo Pump

2.8.3 EGP Econo Gradient Pump

The Econo Gradient Pump (EGP) is a two-channel, bi-directional, variable speed peristaltic pump for low-pressure chromatography and general laboratory use. The EGP has the following features:

- The EGP can be programmed to run a gradient.
- The EGP controls a splitter valve for stream splitting.
- The EGP may be used with most flexible tubing having an inner diameter less than or equal to 3.2 mm (1/8"), and a wall thickness of 1.0 mm or less, including Silicone, Tygon, and PharMed.
- Flow rates are 0.01-20 ml/min per channel, depending upon tubing ID.
- It has start/stop control of fraction collectors and chart recorders.
- It is coldroom compatible.

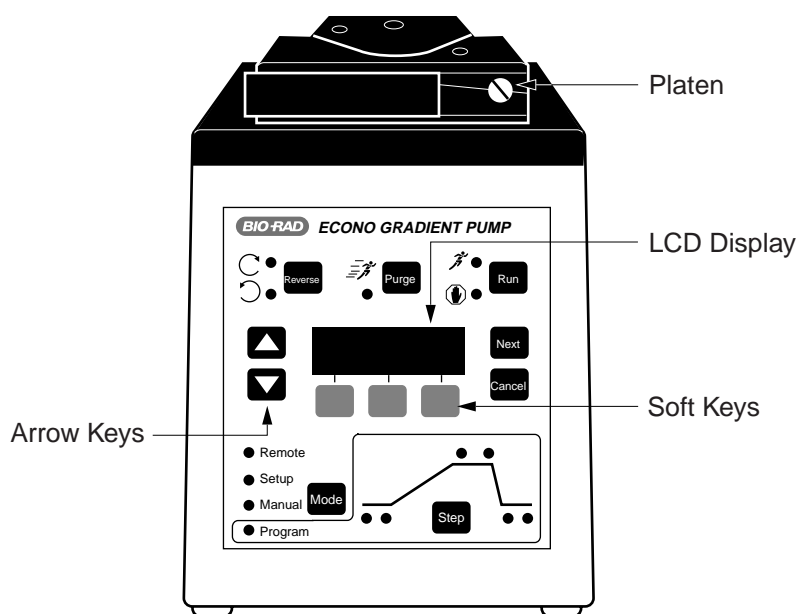


Figure 2-29. EGP Econo Gradient Pump

The EGP is connected to the DuoFlow Workstation with Bus communication cable (System cables 17, 18, 19, or 21). When the DuoFlow assumes control of the EGP, the EGP is automatically set to Remote mode. In Remote mode, the EGP keys provide limited control, allowing only basic observation of EGP operating parameters. For a complete discussion of the EGP, refer to its separate documentation.

The illustration below shows the Aux pump connected to port 3 of the inject valve.

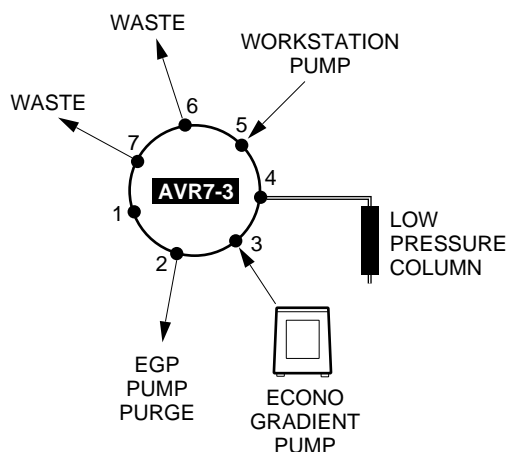


Figure 2-30. Example of Large Sample Loading using an Econo Gradient Pump (EGP)

The Econo Gradient Pump (EGP) can be used to load up to 7 samples sequentially when used with an AVR9-8 at the pump's inlet valve, as shown below.

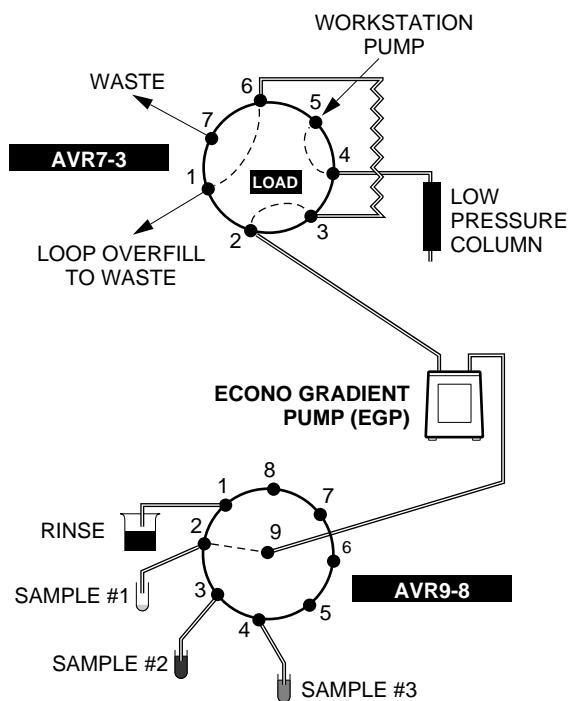


Figure 2-31. Example of Multiple Sample Loading using an Econo Gradient Pump (EGP)

2.8.4 Other Gradient Pumps

The BioLogic DuoFlow system sends a TTL signal to control an external pump. This signal is normally TTL high (5 volts). The DuoFlow system commands the external pump to run by holding Pin 4 "low". Any pump accepting this signal may be used with the DuoFlow system as a sample loading pump. Refer to your pump's separate documentation when connecting the pump to the DuoFlow system.

2.9 SYSTEM PERIPHERALS

A number of system peripherals are available for use with DuoFlow systems. The following system peripherals are discussed in this section:

- System rack
- Starter kit
- Fittings kit
- Fittings tightener
- Backpressure regulator
- SIM
- F10 and F40 Pump kits
- Chart recorders, including the Model 1327 chart recorder
- Uninterruptable power supply (UPS)
- Printers

2.9.1 System Rack

The BioLogic rack is an adaptable and durable racking system made of solvent-resistant polypropylene, stainless steel, and glass-filled nylon. Features of the rack include:

- A rack expansion kit and optional rack components are available for designing a custom rack.
- The rack can stand alone or be placed directly on the Workstation.
- The rack supports various columns and cartridges, valves, detection modules, buffer bottles, and peripheral equipment.

Assemble the rack before placing it on the Workstation, as described below refer to figure 2-32. Keep in mind that the illustration is only an example. Alternative rack arrangements include mounting the column clamping arrangement to the side of the rack to hold tall columns or setting up the rack to use only one or two trays.

1. Fit the tapered collars into the ringed grooves on the rods. The collars are tapered so that when they are attached to the rods and the rods are fitted into the holes at each end of the tray, the rods and collars serve as the legs of the tray. With this in mind, note the following guidelines:
 - For a 2 or 3-tray rack, you will need four long rods; for a 1-tray rack you will need just two long rods. The long rods have three grooves, one at each end and one in the middle. Attach the collars to one of the end grooves. It doesn't matter which of the end grooves you use. Attach the collars so that they flare out toward the closer end of the rod.
 - For a one tray rack, use the two short rods which have only one groove. Fit the collars onto the grooves so that the collars flare out toward the farthest end of the rod.

2. Insert the rods into the holes at each corner of a tray. Insert the rods from *underneath* the tray such that they produce a firm fit in the holes.
3. For a 2 or 3-tray rack, attach sleeves to the middle of all four long vertical bars. Remember that the sleeves should be oriented so that the wide part of the taper is nearest the rod's bottom end.

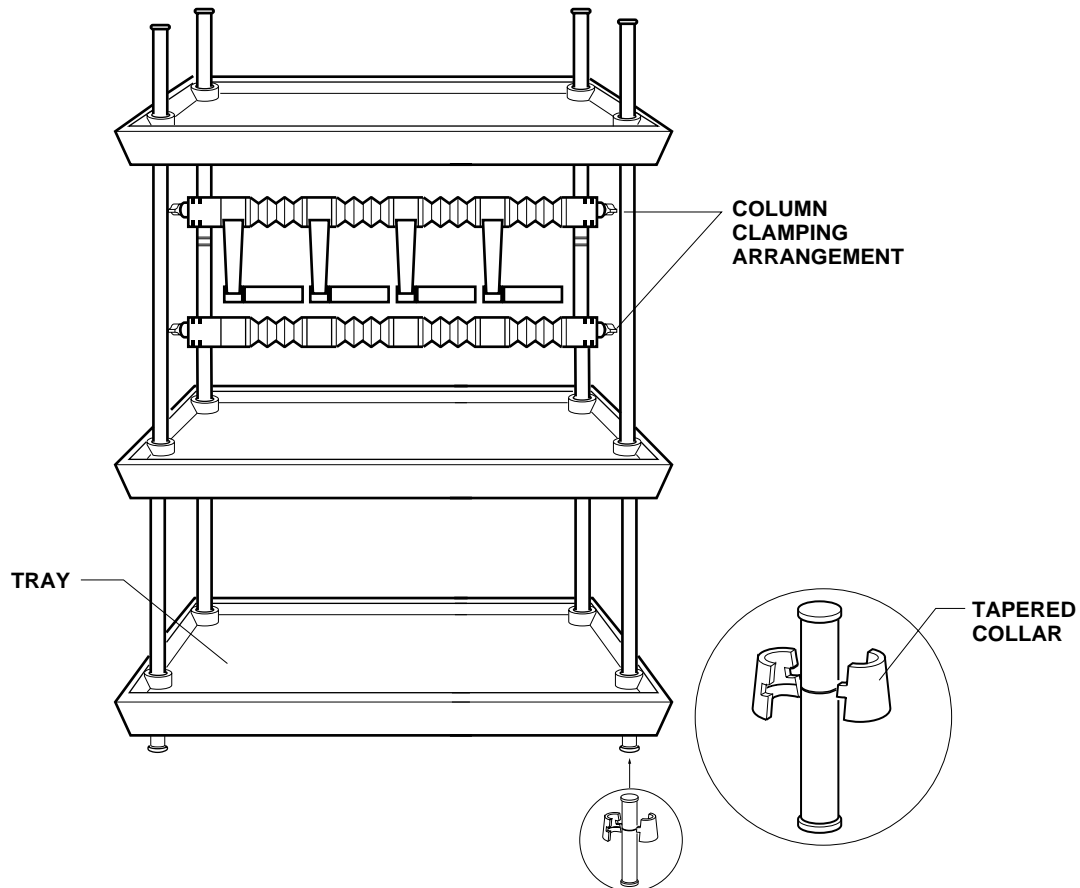


Figure 2-32. Rack Assembly

4. For a 2 or 3-tray rack, place the second tray on top of the four sleeves and press firmly to seat the tray. Repeat these instructions to add the upper tray.
5. Mount the 2-piece column clamping arrangement across the two long rods using the attached thumbscrews.
6. Two horizontal bars with rod clamps and an Allen wrench are provided with the system. These are used to hold devices, such as valves. Position them between any of the upright bars.
7. Remove the four green caps covering the holes at the four corners on top of the Workstation. Place the rack into the four corner holes.

2.9.2 Starter Kit

The Starter kit is included with each DuoFlow system. The kit includes step-by-step procedures for programming and running a separation of a premixed anion exchange standard containing equine myoglobin, conalbumin, chicken ovalbumin, and soybean trypsin inhibitor, using a 1.3 ml UNO Q1 Column.

The Starter kit includes the following items for running a separation:

- 50 ml of Buffer A, 250 mM Tris buffer pH 8.1 (10 X concentrate)
- 50 ml of Buffer B, 250 mM Tris buffer pH 8.1 plus 5.0 M NaCl (10 X concentrate)
- One vial of Anion Exchange Protein Standards (catalog number 125-0561)
- One 1-ml disposable sample injection syringe
- One 50 µl sample loop
- 50 ml of Maximizer solution A1 (10x concentrate)
- 50 ml of Maximizer solution A2 (10x concentrate)
- 50 ml of Maximizer solution B2 (2.5x concentrate)

The UNO Q1 Column (catalog number 720-0001) is not included with the Starter kit but is included with each DuoFlow system.

For a complete discussion of the Starter kit, refer to its separate documentation.

2.9.3 Fittings kit, including Tubing kit

The DuoFlow Fittings kit is included with all DuoFlow system. It contains the following components:

- PEEK and Teflon Tubing
- Tefzel Plugs, Adaptors, Unions, and Caps
- Tubing Cutter
- 3 ml Syringe and 10 ml Luer Slip Syringe
- Molded Bottle Caps
- BioLogic Fittings Tool
- Screwdriver
- PEEK F to M and F to F Luers
- Delrin Nuts
- Super Flangeless Ferrules
- F10 Tubing Kit

Optional tubing kits include the following:

- F10 Tubing Kit

The F10 Tubing Kit includes cut and 1/4-28 fitted Teflon, Tefzel, and 0.02" ID PEEK tubing for easy installation of a DuoFlow basic systems. An installation chart indicates suggested placement of each piece of labeled tubing. (Refer to page 4-1)

The kit includes: a length of Teflon tubing, 1/8" OD, for connecting solution bottles to the Workstation pumps A and B; a length of Tefzel tubing, 1/16" OD, for connecting AVR7-3 waste lines; and 8 additional pieces of PEEK, orange, 1/16" OD x 0.02" ID tubing, to connect the pump, mixer, column, detector, and other components.

- F40 Tubing kit

The F40 Tubing kit is identical to the F10 kit (described previously), however, all orange PEEK tubing is replaced with PEEK, green, 1/16" OD x 0.03" ID tubing. The large bore tubing is designed for use with the higher flow rates when using the Maximizer or F40 Workstation pumps.

- Maximizer Tubing kit

The tubing kit that accompanies the Maximizer for connecting the reagent bottles to the valves is composed of FEP Teflon, 1/8" OD x 0.062" ID. Each length of tubing is color coded to identify its connection site and solution.

Inlet A1 uses red tubing and is used for acid solutions. Inlet A2 uses blue tubing and is used for base solutions. Inlet B1 uses yellow tubing and is used for water solutions. Inlet B2 uses green tubing and is used for salt solutions.

The kit includes an installation chart.

- Maximizer Interconnect Tubing kit

This kit contains cut and fitted tubing to connect the Workstation pumps A and B inlets to the Maximizer valves A and B outlets. There are two preformed PEEK 1/8" OD sets of tubing in this kit.

- pH Montior Tubing kit

The pH monitor includes cut and fitted tubing to connect the pH flow cell to the Conductivity flow cell. There is a length of PEEK, orange, 1/16" OD x 0.02" ID tubing rated to 5000 psi and a length of PEEK, green, 1/16" OD x 0.03" ID tubing rated to 3000 psi for higher flow rates.

2.9.4 Fittings Tightener

The fittings tightener is designed to apply appropriate tightness to the nut, stainless steel lock ring, and the ferrule, when installing 1/4-28 fittings on the end of tubing. The flattened end of the lock ring should face towards the nut with the tapered end facing the tapered end of the brown ferrule. Place the tubing and fittings into the green fittings tightener. Do not allow the tubing to slip out of the ferrule. Tighten the fitting to seat the ferrule onto the tubing, but do not over-tighten. Once the fitting is made, it can be inserted into the port. Refer to instructions on next page.

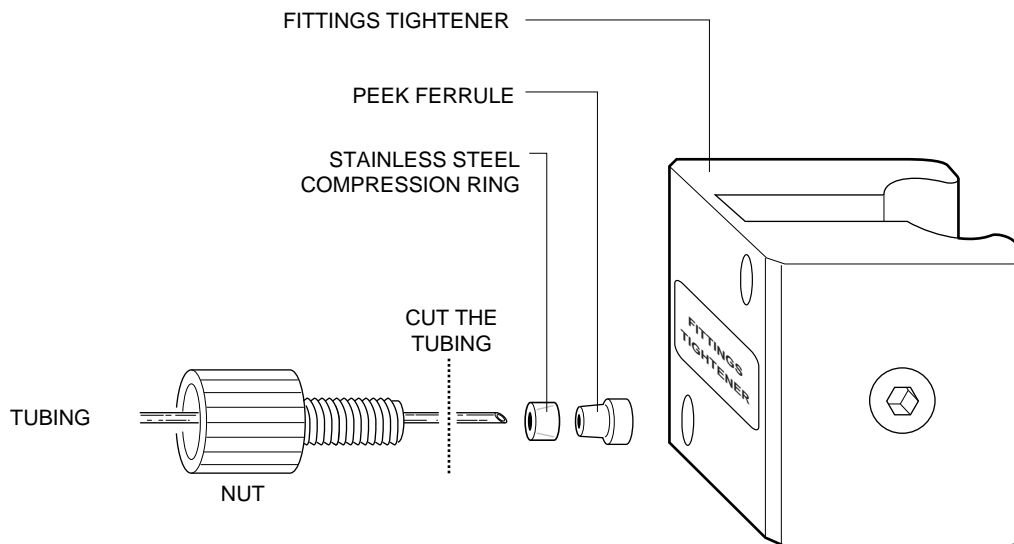


Figure 2-33. Making 1/4-28 Flat Bottom Fittings

The following procedure describes ferrule installation.

1. Slide the nut, stainless steel compression ring and the ferrule, in that order onto the tubing, as shown at left. The flattened end of the ring should face the nut. The tapered end of the ferrule should face the ring.
2. Allow tubing to extend slightly beyond the end of the ferrule.
3. Place the fitting into the green fittings tightener. Do not allow the tubing to slip out of the ferrule.
4. Insert the tubing and fitting into the fittings tool to tighten to seat the ferrule onto the tubing, do not overtighten.

2.9.5 Backpressure Regulator

The 40 psi backpressure regulator is used with flow rates below 10 ml/min. Plumb the backpressure regulator following the direction of the arrow.

The Backpressure Regulator helps eliminate bubble information within the detector. As a solution is pumped through a column, the column exerts a backpressure that serves to keep any air bubbles in solution. Solution exiting the column returns to atmospheric pressure and air bubbles may form. As the bubbles pass through, or lodge in the detector flow cell they may cause artifacts on the baseline chromatogram that appear as spikes. This “outgassing” may be minimized by thoroughly degassing buffers and by placing a backpressure regulator after the Conductivity monitor. The backpressure from the regulator helps to keep the bubbles in solution.

When using low pressure columns such as an Econo-Pac® cartridge, plumb the 40 psi backpressure regulator between the Workstation pump outlet and the mixer. This aids in seating the check valves, preventing permanent damage to the cartridge or column.

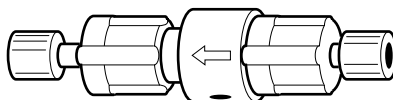


Figure 2-34. Backpressure Regulator

2.9.6 Signal Import Module (SIM)

The Signal Import Module digitizes the analog signal and transmits it to the DuoFlow Controller enabling additional detectors to be used with the DuoFlow.

- The SIM allows for connection of both a pH monitor and other detector which outputs an analog signal between -2.5 Volts to +2.5 Volts. Instruments that may be connected in this way could include a variable wavelength UV detector, a refractive index detector, or a fluorescence detector.
- Two SIMs may be connected to a DuoFlow system.

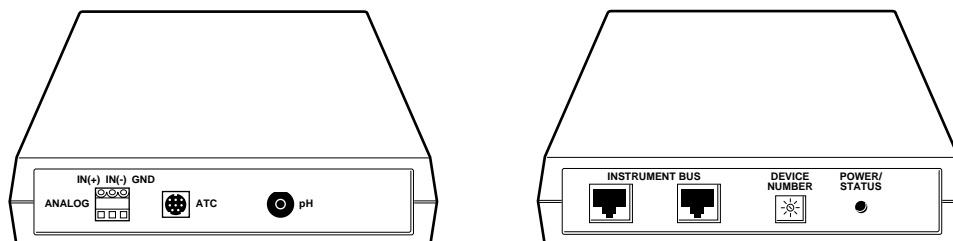


Figure 2-35. Signal Import Module (front and rear views)

The output from a pH electrode is connected to the pH connector (a BNC connector) on the SIM; the output from any other detector is connected at the 3-pin connector (+, -, gnd) of the SIM. The Automatic Temperature Compensation (ATC) connector is reserved for future upgrades and enhancements.

The instrument bus cable connects the SIM to the USB Bitbus Communicator. (Refer to Figures 2-35 and 2-36.) If a second SIM is to be used, one of the instrument bus cables is used to connect the two SIMs. The SIM is described in detail in its separate documentation.

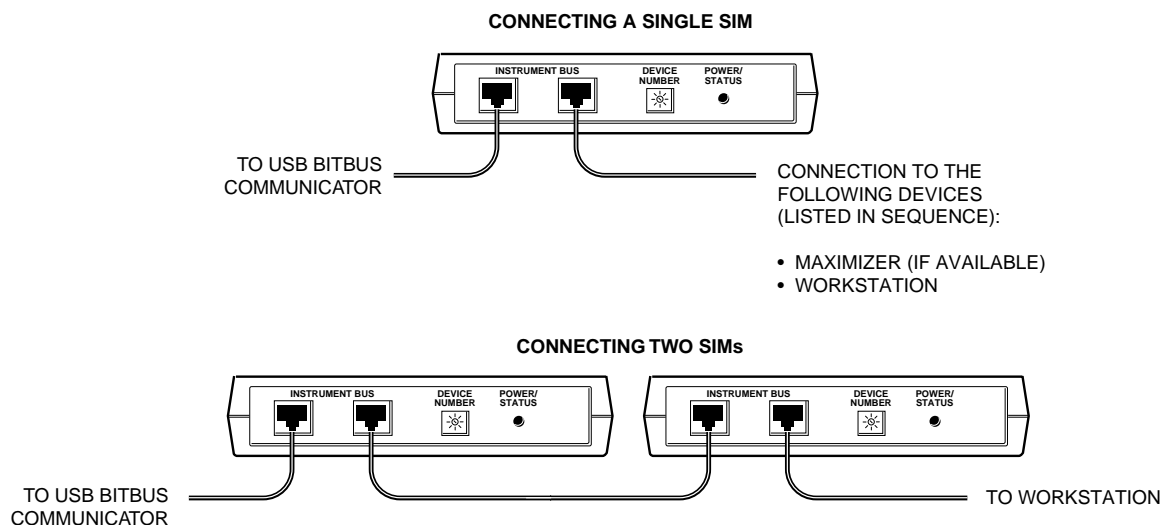


Figure 2-36. Cable Connections to the Signal Import Module

2.9.7 Pump Kits

The Workstation pumps are easily converted to either a F10 or F40 pumphead to expand functional pump flow rates as indicated in the table below.

Table 2-13.
Workstation Pump Configuration Flow Rates

Workstation Pump	Flow Rate	Flow Rate with Maximizer
F10	0.01-10 ml/min	0.5-20 ml/min
F40	0.5-40 ml/min	1.0-80 ml/min

There are two kits, the F10 Pump kit and the F40 Pump kit. Each kit contains fully assembled pumpheads with seals and check valves installed.

The F10 Pump kit contains the following items:

- Two F10 pumpheads assembled with check valves, seals, and O-rings
- Four F10 piston assemblies
- Installation instructions

PEEK tubing, orange, 1/6" OD x 0.02 ID is supplied in the Fittings kit of the DuoFlow system

The F40 Pump kit contains the following items:

- Two F40 pumpheads assembled with check valves, seals, and O-rings
- Four F40 piston assemblies
- One 2 ml mixer barrel extender
- Two mixer O-rings
- One 2mm UV flow cell
- PEEK tubing, green, 1/16" OD x 0.03" ID (rated to 3000 psi)
- Installation instructions

For a complete discussion of the Pump kits, refer to the separate documentation supplied with the kits.

2.9.8 Model 1327 Chart Recorder

The Model 1327 chart recorder is a dual-pen chart recorder that is compatible with many detection devices. The chart recorder includes the following features:

- Two-channel, dual-pen capability.
- Compact in size (31 x 23 x 7.6 cm).
- Voltage ranges from 1 mV to 20V.
- Twelve chart speeds for recording methods.

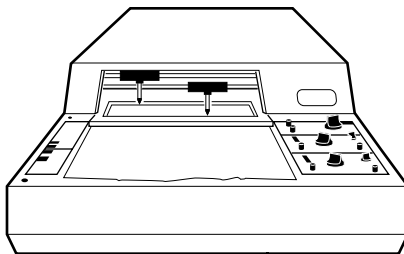


Figure 2-37. Model 1327 Chart Recorder

The DuoFlow Controller outputs the UV analog data signal, pen up/down, and Stop/Start commands to the Model 1327 chart recorder via the Workstation's UV Chart connector (cabled to the chart recorder using **System Cable 2** mini DIN to standard DIN). The Conductivity analog data signal is sent from the Workstation's Conductivity Chart (**Cond Chart**) connector to channel 2 of the chart recorder using **System Cable 4** (mini-DIN to banana plugs). The chart recorder should be set to all green settings (1V). The chart recorder is described in detail in its separate documentation.

Note: The chart speed is set at the recorder. The BioLogic DuoFlow does not control this function.

Event marks are recorded on the chart recorder for the following:

- Fraction Collector Advance: When the fraction collector advances to the next tube.
- A manual event mark from the Run screen. Pressing the **Event Mark** button on the Run screen allows you to "mark" events as they happen.

Non-Bio-Rad UV detectors: If the Model 1327 chart recorder is used in conjunction with a SIM and a third-party detector to replace the DuoFlow UV Detector, **System Cable 20** should be used to control the recorder. Channel 1 signals should be sent directly from the third-party detector using a bare wires-to-banana plug cable. In this case, the appropriate input voltage range must be selected on the recorder. Refer to the documentation for your non-Bio-Rad UV detector.

2.9.9 Generic Chart Recorders

A non-Bio-Rad chart recorder may be used as an integral part of the DuoFlow system. If the DuoFlow system's UV detector is used, a mini-DIN to breakout cable (**System Cable 7**) must connect the Workstation's UV Chart connector to the chart recorder. Pen Up/Down and Start/Stop functions are available providing the control polarity of the generic recorder is compatible. See the pin-out information in your chart recorder manual.

Conductivity signals require a Bio-Rad mini-DIN to banana plug cable (**System Cable 4**).

Set the chart recorder's input signal voltage to 1 V for both BioLogic DuoFlow UV detector and Conductivity monitor signals. Chart speed is set at the recorder itself, it is not controlled by the DuoFlow system.

2.9.10 Uninterruptible Power Supply (UPS)

A UPS may be required in laboratory environments that experience power outages or where the quality of power varies. Bio-Rad can supply a UPS in both 110 V and 220 V configurations. For questions about this UPS, consult your local Bio-Rad representative.

2.9.11 Printers

Any Windows® 2000 compatible printer may be used with the DuoFlow systems. The printer must include a connection cable and a printer driver.

To install a printer driver, exit the DuoFlow software. If the printer driver disk is available, follow the instructions that are included with that disk. If the printer driver disk is not available, click on the Windows® 2000 **Start** button and from **Settings**, select **Control Panel**.

In the Control Panel, double-click on the **Printers** icon to open the Printers window. Then double-click on the **Add Printer** icon and follow the on-line instructions. If your printer does not appear in the list of printers available, contact the printer manufacturer for a recommendation on which driver to use.

2.10 COLUMNS AND COLUMN FITTINGS

Bio-Rad offers a variety of column chemistries and formats. The following pages provide a summary of the different column types and column fittings for use with BioLogic DuoFlow systems. Bio-Rad columns for the DuoFlow use the following matrices:

- UNO™ columns use the new Continuous Bed matrix which contains an advanced polymer matrix that is completely homogeneous. These columns are superior to beaded supports in resolution, binding capacity, speed, and value.
- Bio-Scale chemistries are based on 10 µm Macro-Prep® supports. They are offered in various sizes and allow for scale-up separation and purification. These medium-pressure columns are ideally suited for use on the DuoFlow system.
- The Econo-Pac cartridge chemistries are based on 50 µm Macro-Prep® supports and are ideal for first step purification.

2.10.1 Anion Exchange: Q Strong Anion Exchange

The Q strong anion exchanger chemistry is available in the following formats:

- **UNO Q Biochromatography Columns:** These columns are designed to handle separations at high flow rates with low back pressure. Instead of a traditional bed of packed beads or particles, each column contains an advanced polymer matrix, called the Continuous Bed matrix, which is nonporous and homogeneous. The matrix is designed to maximize resolution, binding capacity, and speed. Refer to bulletins 2116 and 1946.
- **Bio-Scale Q Prepacked Medium Pressure Columns:** These columns are designed for high resolution separations of proteins, peptides, and polynucleotides in analytical to semipreparative medium pressure applications. They are available in four column sizes. Methods developed on the Bio-Scale columns can be transferred to production scale using the Macro-Prep® supports. Refer to bulletins 1880, 1946, and 2079.
- **Econo-Pac Q and High Q Low Pressure Chromatography Cartridges:** These cartridges are available in 1 ml and 5 ml formats to accommodate most sample loads. They are recommended for method scouting and for first step purification of crude samples. They are based on 50 µm Macro-Prep® supports. Refer to bulletin 1946.
- **Macro-Prep® Q and High Q Support:** This is a strong anion exchanger containing quaternary amine functional groups with a 50 µm particle size. It is ideal for rapid purification of acidic and neutral proteins and peptides. Refer to bulletins 1840 (A-100), 1917, and 1985.

2.10.2 Cation Exchange: S Strong Cation Exchange

The S strong cation exchanger chemistry is available in the following formats:

- **UNO S Biochromatography Columns:** These columns are designed to handle separations at high flow rates with low back pressure. Instead of a traditional bed of packed beads or particles, each column contains an advanced polymer matrix, called the Continuous Bed matrix, which is nonporous and homogeneous. The matrix is designed to maximize resolution, binding capacity, and speed. Refer to bulletins 2116 and 1946.
- **Bio-Scale S Pre-packed Medium Pressure Columns:** These columns are designed for high resolution separations of proteins, peptides, and polynucleotides in analytical to semipreparative medium pressure applications. They are available in four column sizes. Methods developed on the Bio-Scale columns can be transferred to production scale using the Macro-Prep® 50 µm supports. Refer to bulletins 1881, 1946, and 2079.

- **Econo-Pac S Low Pressure Chromatography Cartridges:** These cartridges are available in 1 ml and 5 ml formats to accommodate most sample loads. They are recommended for method scouting and for first step purification of crude samples. They are based on 50 µm Macro-Prep® supports. Refer to bulletin 1985.
- **Macro-Prep® High S Support:** This is a strong cation exchanger containing sulfonic acid functional groups with a 50 µm particle size. It is ideal for purification of basic and neutral proteins and peptides. Refer to bulletins 1840 (A-200), 1917, and 2079.

2.10.3 Anion Exchange: DEAE Weak Anion Exchange

The DEAE weak anion exchanger chemistry is available in the following formats:

- **Bio-Scale DEAE Prepacked Medium Pressure Columns:** These columns are designed for high resolution separations of proteins, peptides, and polynucleotides in analytical to semipreparative medium pressure applications. They are available in four column sizes. Methods developed on the Bio-Scale columns can be transferred to production scale using the Macro-Prep® 10 µm supports. Refer to bulletins 1930, 1946, and 2079.
- **Econo-Pac DEAE Blue Low Pressure Chromatography Cartridges:** These cartridges are available in 1 ml and 5 ml formats to accommodate most sample loads. They are recommended for method scouting and for first step purification of crude samples. They are based on 50 µm Macro-Prep® supports. Refer to bulletin 1946.
- **Macro-Prep® DEAE Support:** This is a weak anion exchanger containing diethylaminoethyl functional groups with a 50 µm particle size. It is ideal for purification of acidic and neutral proteins and peptides. Refer to bulletin 1840 (A-400).

2.10.4 Cation Exchange: Carboxy Methyl (CM) Weak Cation Exchange

The CM weak cation exchanger chemistry is available in the following formats:

- **Econo-Pac CM Low Pressure Chromatography Cartridges:** These cartridges are available in 1 ml and 5 ml formats to accommodate most sample loads. They are recommended for method scouting and for first step purification of crude samples. They are based on 50 µm Macro-Prep® supports.
- **Macro-Prep® High CM Support:** This is a weak cation exchanger containing carboxylate functional groups with a 50 µm particle size. It is ideal for purification of basic and neutral proteins and peptides. Refer to bulletins 1840 (A-300) and 1946.

2.10.5 Ceramic Hydroxyapatite (CHT)

The CHT chemistry is available in the following formats:

- **Bio-Scale CHT Type I Prepacked Medium Pressure Columns:** These columns are designed for high resolution separations of proteins, peptides, and polynucleotides in analytical to semipreparative medium pressure applications. They are available in four column sizes. Methods developed on the Bio-Scale columns can be transferred to production scale using the Macro-Prep® 10 µm supports. Refer to bulletins 1929, 1946, 2079, and 2156.
- **Econo-Pac CHT-II Low Pressure Chromatography Cartridges:** These cartridges are available in 1 ml and 5 ml formats to accommodate most sample loads. They are recommended for method scouting and for first step purification of crude samples. They are based on 20 µm Macro-Prep® supports. Refer to bulletin 1946.

- **Macro-Prep® CHT Support:** Macro-Prep® ceramic hydroxyapatite (CHT) overcomes the physical and chemical instability of crystalline hydroxyapatite and is available in two types. Type I has a high protein binding capacity and better binding capacity for acidic proteins. Type II is better suited for proteins that elute early and for nucleic acids. Refer to bulletins 1842 (C-100), 1927, 1971, and 2156.

2.10.6 Size Exclusion Chromatography (SEC)

The SEC supports are available in the following formats:

- **Macro-Prep® SE Support:** Macro-Prep SE 100/40 and SEC 1000/40 gels are used for applications ranging from the separation of protein aggregates (dimers, trimers, etc.) from purified antibody solutions to the separation of complex mixtures of bio-molecules directly from cell culture feedstreams. SEC is commonly used as a final polishing step in the preparation of many bio-pharmaceuticals and has numerous applications as a quality control tool for assessing product purity and concentrations. These gels are made from biocompatible 40 µm agarose beads. Refer to bulletin 2190.
- **HPLC SEC Columns:** Bio-Sil and Bio-Select 5 µm silica-based columns separate compounds by the mechanism of size exclusion. The technique is based on diffusion in and around highly porous spherical silica beads. The columns are recommended for the separation of peptides, proteins, and nucleic acids, for desalting or buffer exchange, and for molecular weight or molecular constant determination. Columns are available in both stainless steel (Bio-Sil columns) and biocompatible PEEK plastic hardware (Bio-Select columns). Each column is shipped with a free vial of Bio-Rad's protein standards. Refer to bulletins 1737 and 1946.
- **Econo-Pac P6 Low Pressure Chromatography Cartridges:** This 5 ml cartridge is used for desalting and buffer exchange. Refer to bulletin 1946.

2.10.7 High Pressure Reversed Phase Columns

The following type of column is available:

- **Hi-Pore reversed phase columns:** These columns are commonly used for the purification and analysis of small proteins (<50 kd), peptides, oligonucleotides, and amino acids. Refer to bulletin 1946.

2.10.8 Hydrophobic Interaction Chromatography (HIC)

The HIC chemistry is available in the following formats:

- **Econo-Pac t-Butyl HIC Low Pressure Chromatography Cartridges:** These cartridges are available in a 5 ml format to accommodate most sample loads. They are recommended for method scouting and for first step purification of crude samples. They are based on 50 µm Macro-Prep® supports. Refer to bulletins 1946 and 2079.
- **Econo-Pac HIC Low Pressure Chromatography Cartridges:** HIC is offered in both methyl and t-butyl chemistries. These cartridges are available in 1 ml and 5 ml formats to accommodate most sample loads. They are recommended for method scouting and for first step purification of crude samples. They are based on 50 µm Macro-Prep supports. Refer to bulletin 1946.
- **Macro-Prep® HIC Support:** HIC is offered in both methyl and t-butyl chemistries. The Macro-Prep® methyl HIC support is ideal for purification of proteins with strongly hydrophobic regions. The Macro-Prep® t-butyl HIC support is ideal for purification of proteins with few or weakly hydrophobic regions. Refer to bulletin 1841.

2.10.9 Affinity Chromatography

Affinity chromatography is available in the following formats:

- **Econo-Pac Protein A Low Pressure Chromatography Cartridges:** These cartridges are available in 1 ml and 5 ml formats to accommodate most sample loads. They are recommended for monoclonal antibody purification. Refer to bulletin 1946.
- **Econo-Pac Heparin Low Pressure Chromatography Cartridges:** This is available in a 5 ml format for purification of a range of proteins. Refer to bulletin 1946.
- **Econo-Pac Blue and DEAE Blue Low Pressure Chromatography Cartridges:** These chemistries are available in a 5 ml format and are recommended for albumin and protease removal. Refer to bulletin 1946.

2.10.10 Empty Columns

The following columns are available:

- **Bio-Scale MT empty columns:** These columns allow for easy packing of a chromatographic media, bed height adjustment, sample application, and equilibration. The availability of four column sizes (2, 5, 10, and 20 ml) allows easy scale-up of separation and purification protocols. Request bulletin 1970.
- **Glass Econo-Column columns:** Econo-Column chromatography columns are the standard for high quality, affordable low pressure chromatography columns. They accept both Econo-Column funnels and flow adapters.

2.10.11 Column Fittings

Table 2-14.
Columns and Column Fittings

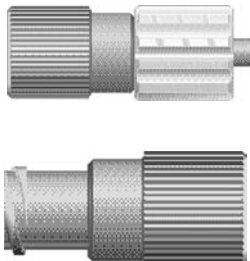



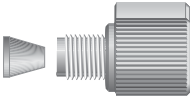
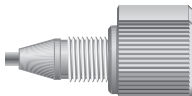
Column Type	Description	Fittings Required
Econo-Pac® Cartridge	The Econo-Pac cartridge has one male and one female luer-lock fitting. To connect it to the BioLogic system use 1/4-28 to male and 1/4-28 to female luer adapters, provided in the kit shown to the right. One set is included in the Fittings kit.	<p>Catalog # 732-0113 Econo-Pac® Cartridge to Fittings Kit</p> 
Econo-Column®/ Low Pressure Columns and flow adapters	<p>Econo-Columns have two male luer-lock fittings. To connect to the system, use female luer to 1/4-28 adapters.</p> <p>If a flow adapter with flexible tubing is to be used, attach the barbed-to-male luer fitting (included in this kit), then connect the female luer to 1/4-28 adapter.</p> <p>If the column adapter uses Teflon® tubing, attach the small length of 1/16" (1.6 mm) ID Tygon® tubing and insert the barbed male luer fitting into open end. Five cm of Tygon tubing is included in this kit. Connect as above.</p>	<p>Catalog # 750-0565 Econo-Column® to BioLogic System Fittings Kit</p>  <p>This kit includes all the parts needed to connect Econo-Columns and flow adapters to the BioLogic DuoFlow system.</p>
FPLC® Column	Pharmacia's FPLC columns use M-6 fittings. To connect to the system, use 1/4-28 to M6 adapter union. Two of these are supplied with the Fittings kit.	<p>Catalog # 750-0561 Union, 1/4-28 to M6</p> 

Table 2-14. (continued)
Columns and Column Fittings

Column Type	Description	Fittings Required
HPLC Column, or Pharmacia's RESOURCE™ Column	These columns accept 10/32 nuts. To connect this type of column to the system, use two 1/4-28 to 10-32 adapters.	Catalog # 750-0564 HPLC Column to BioLogic System Adapters 
UNO™ Column to FPLC system	This kit includes two nuts and four ferrules to connect an UNO column to an FPLC system.	Catalog #750-0567 M6 Fittings Kit 
UNO™ Column to HPLC system	This kit includes two nuts and four ferrules to connect an UNO column to an HPLC system.	Catalog #750-0568 10-32 Fittings Kit 

3.0 SYSTEM SETUP

The modular design of BioLogic DuoFlow systems permits you to arrange your system to best meet your application and space requirements. Each of the following subsections discusses how to set up the different components. Setup of each component is the same, regardless of the system you have.

The Workstation and the Maximizer have many rear panel connectors that are identical. The Maximizer connectors should be used before using those on the Workstation. The conductivity monitor must be connected to the Maximizer.

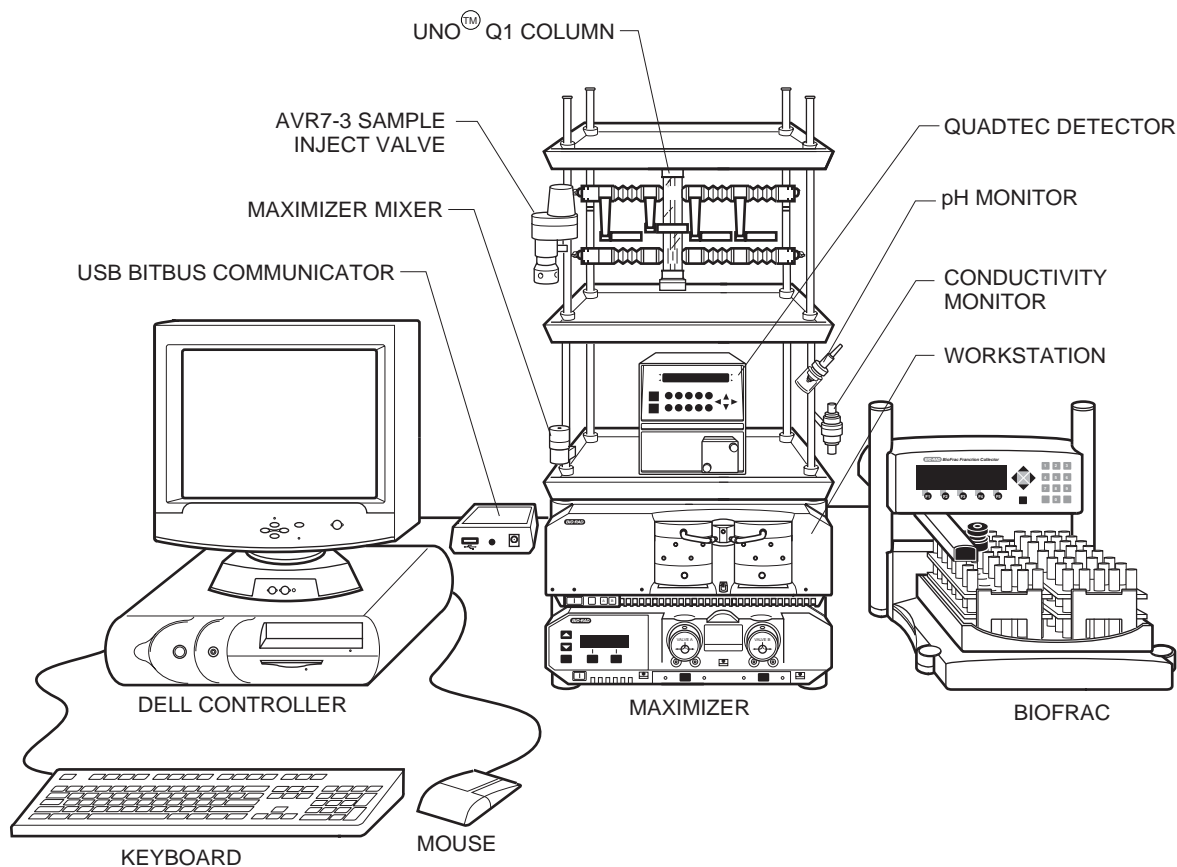


Figure 3-1. Example of a DuoFlow Pathfinder System Configuration

The compact design of the Workstation and rack are ideal for laboratories with limited space or for systems that must go into a cold box or cold room. For these instances the Controller can be placed away from the Workstation (see Figure 3-2 below). Bus communication cables of lengths up to 100 feet can be purchased from Bio-Rad, and USB cables can be purchased from any computer supply store.

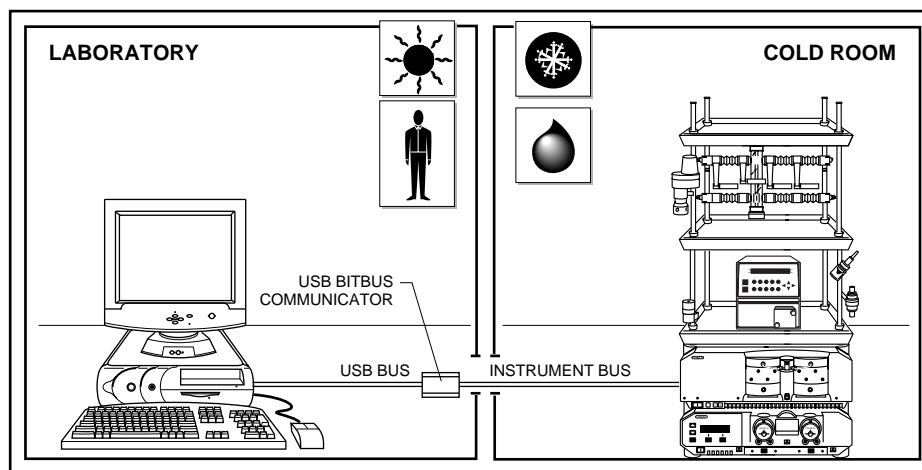


Figure 3-2. DuoFlow Pathfinder Setup in Non-Condensing Environment

3.1 CONTROLLER CABLE CONNECTIONS

The Controller consists of a monitor, computer, keyboard, and mouse. Bio-Rad provides a Dell computer for use as a Controller.

Detailed instructions for setting up your Controller are provided in the separate documentation provided with your Controller.

1. Place the Controller so that the back side is facing you. Connect the monitor, keyboard, and mouse to the computer.
2. Connect the power cables to the computer and monitor. If the computer has a second power outlet, use it to plug in the monitor power cable. **Do not turn on power to these devices yet.**

3.2 USB BITBUS COMMUNICATOR CABLE CONNECTIONS

The USB Bitbus Communicator allows communication between the Controller and the rest of the DuoFlow system. To connect the USB Bitbus Communicator:

1. Connect System Cable **31** from the USB Bitbus Communicator to the computer USB connector. Refer to the illustration below.
2. If a Signal Import Module (SIM) is to be used with the system,
 - a. Connect the USB Bitbus Communicator power adapter.
 - b. Set the Power Select switch to External.

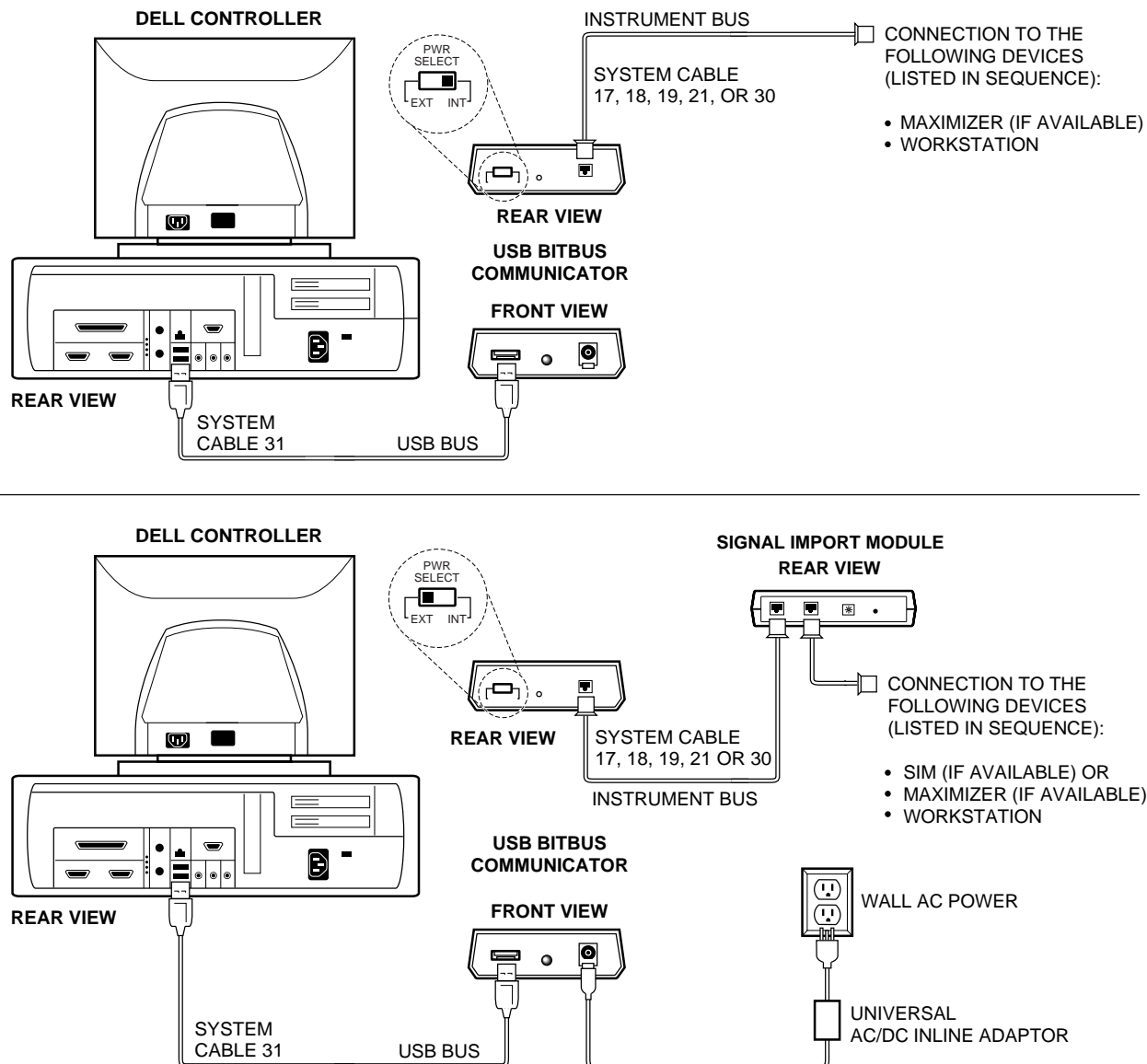


Figure 3-3. USB Bitbus Communicator Cabling

3.3 WORKSTATION CABLE CONNECTIONS

1. Confirm the voltage setting for the Workstation power supply. A red switch on the back of the Workstation allows you to switch between 110 VAC and 240 VAC.
2. Connect the power cable to the Workstation. **Do not turn on this device yet.**

3.3.1 Systems *without* a Maximizer

If the Maximizer is NOT to be part of the system, follow the procedure below to connect the Workstation to the USB Bitbus Communicator.

1. Note the two connectors marked "Instr Bus." These connectors are identical: either may be used when connecting bus communication cables.
2. Select a System Cable of sufficient length to reach the USB Bitbus Communicator. System Cables 17, 18, 19, 21, and 30 differ only in their lengths.

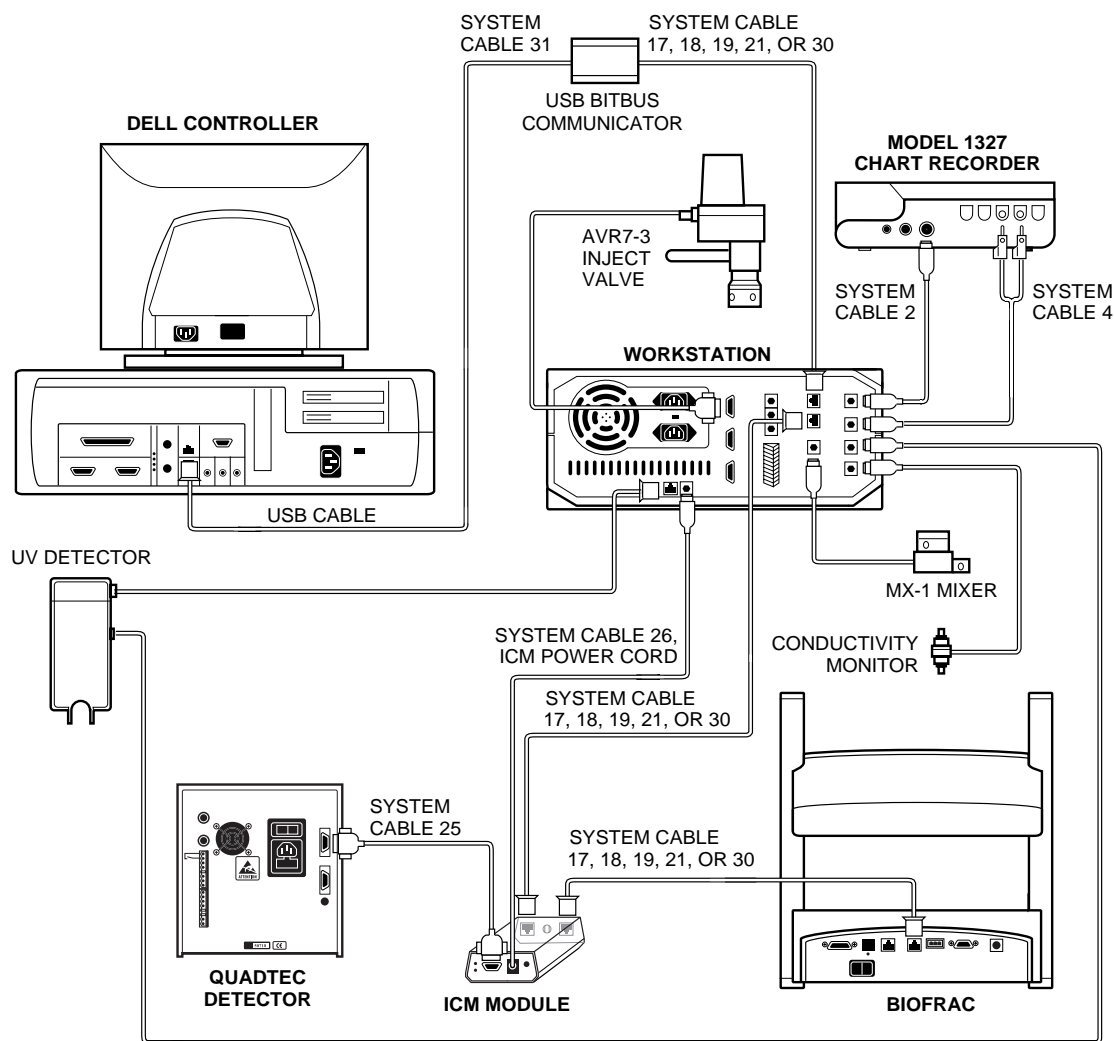


Figure 3-4. System Cable Connections (without Maximizer)

3.3.2 Systems with a Maximizer

If the Maximizer is to be part of the system, follow the procedure below to connect the Maximizer to the Workstation and the USB Bitbus Communicator.

1. Place the Workstation on top of the Maximizer.
2. Note the two connectors marked "Instr Bus." These connectors are identical: either may be used when connecting instrument bus cables.
3. With the back side of the Workstation and Maximizer facing you, connect **System Cable 30** between the Workstation and the Maximizer.
4. Select a System Cable of sufficient length to reach the USB Bitbus Communicator. System Cables 17, 18, 19, 21, and 30 are different only in their lengths.
5. Connect the power cable to the Maximizer. **Do not turn on this device yet.**

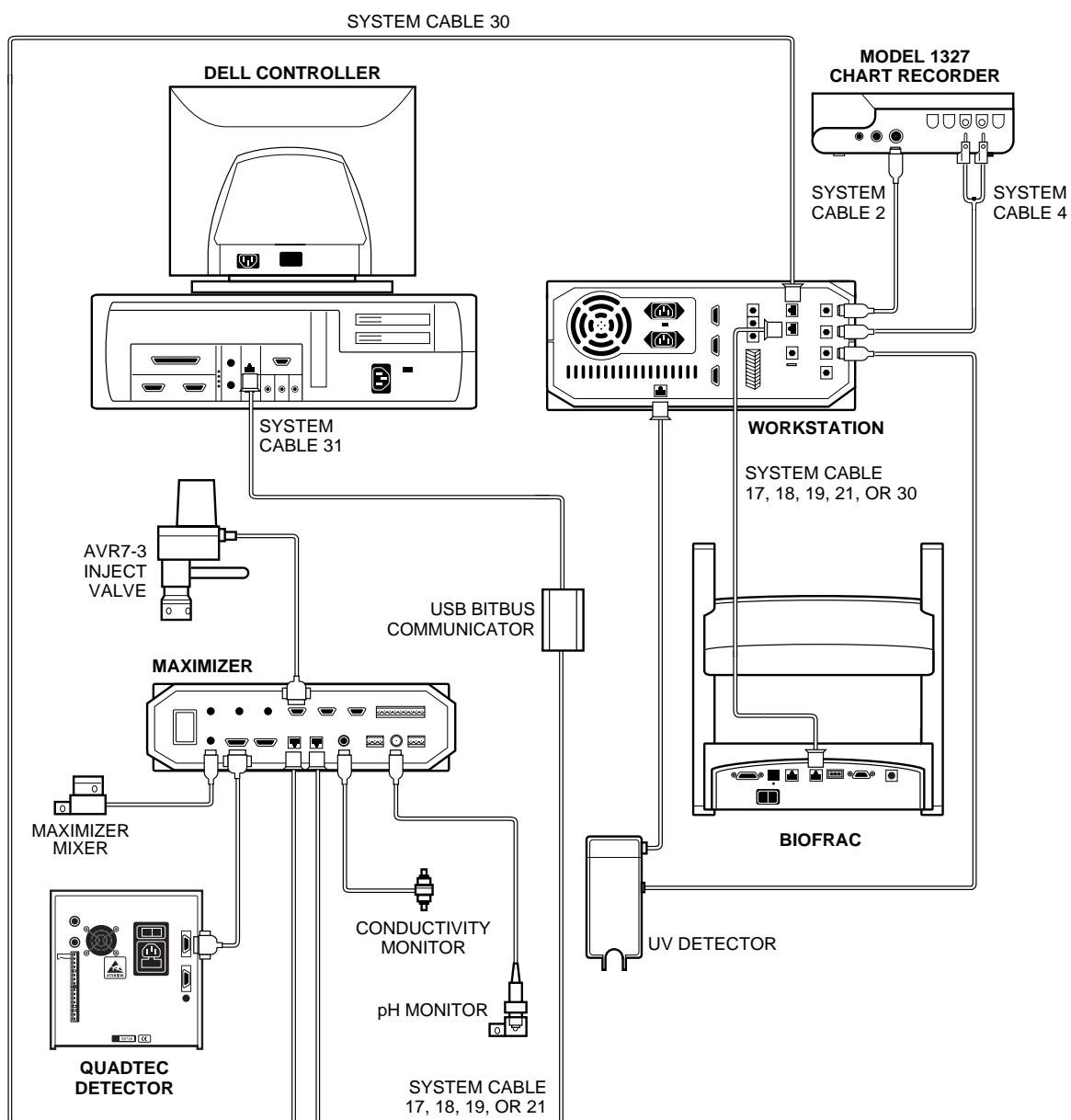


Figure 3-5. System Cable Connections (with Maximizer)

3.4 SYSTEM RACK SETUP

Assemble the system rack before placing it on the Workstation. Detailed discussion of system rack assembly is provided on page 2-48 and 49. Keep in mind that the illustration is only an example. Alternative rack arrangements include setting up the rack to use only one or two trays.

To mount the system rack on the Workstation, remove the four green caps covering the holes at the four corners on top of the Workstation. Place the rack into the four corner holes.

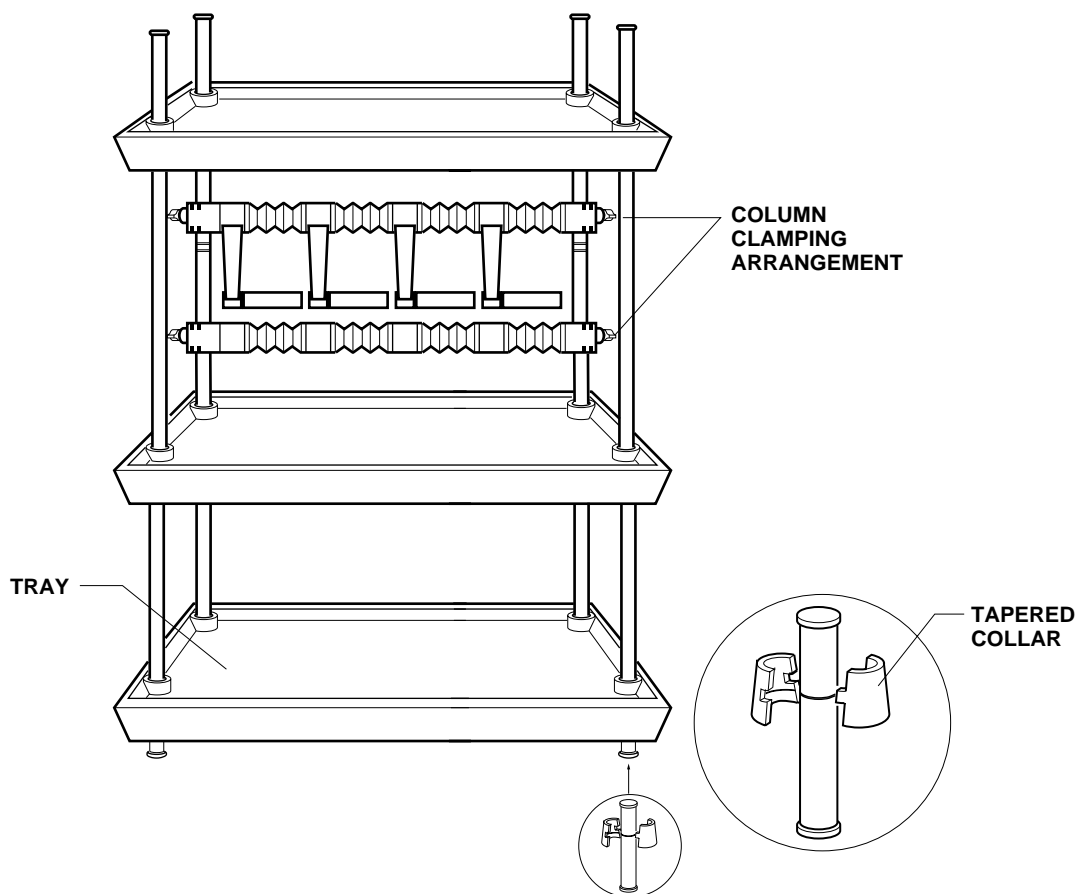


Figure 3-6. Rack Assembly

3.5 MIXERS

Two mixers have been designed for use with the DuoFlow system: the MX-1 mixer and the higher capacity Maximizer mixer. The mixers are shipped pre-assembled with their mixer barrel. The mixer barrel may be removed or replaced increasing or decreasing the internal volume to provide the mixer capacity appropriate to the flow rate. Refer to table 3.1 when selecting a mixer volume.

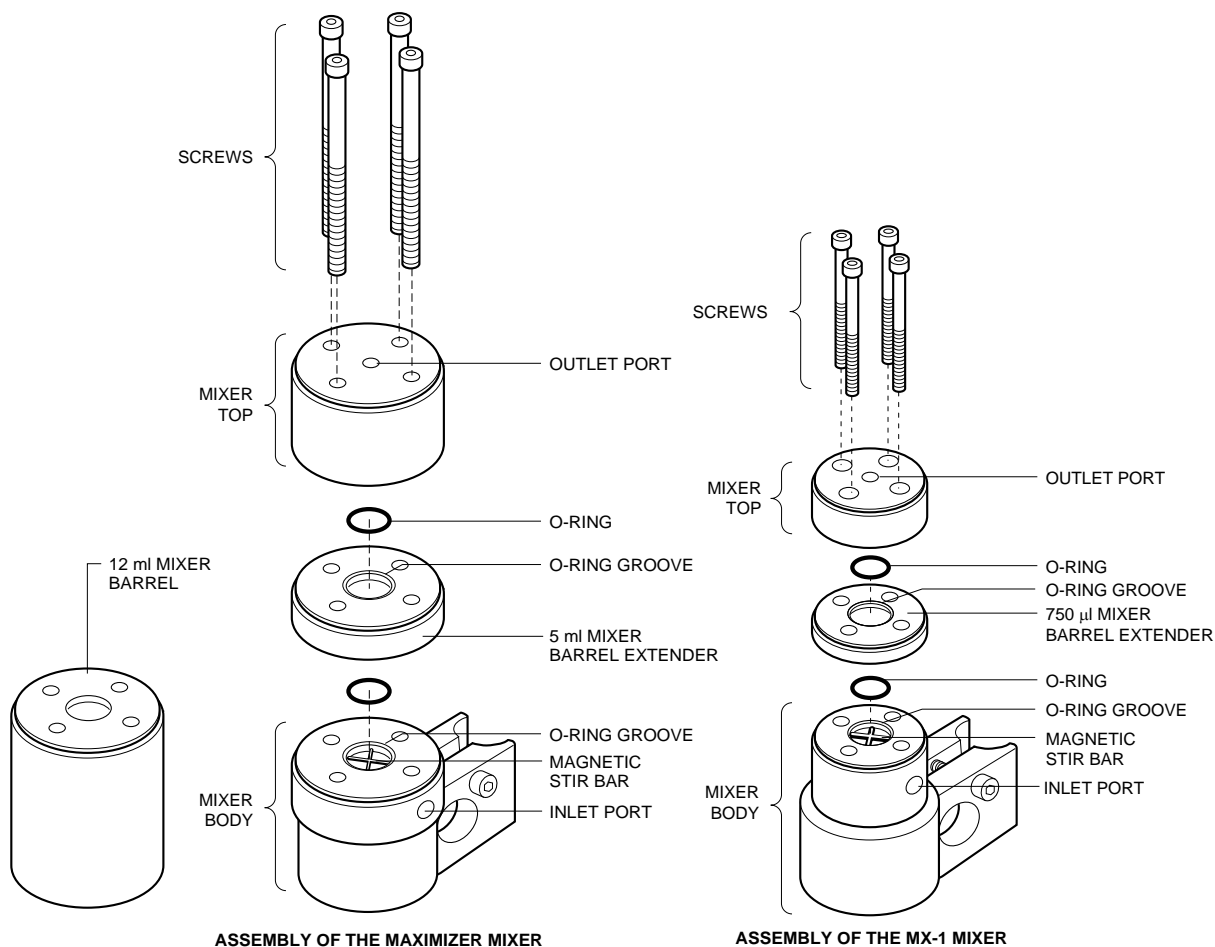


Figure 3-7. Mixers

Table 3-1.
Mixer Flow Rates

Flow Rate	Barrel Extension	Capacity	Assembly Screws
MX-1 Mixer (for use without Maximizer)			
less than 1ml/min	none	263 µl	10-32 x 5/8" (1.6 cm)
1 to 10 ml/min	750 µl mixer barrel extender	750 µl	10-32 x 7/8" (2.2 cm)
10 to 40 ml/min	2 ml mixer barrel extender	2 ml	10-32 x 1-1/2" (3.8 cm)
Maximizer Mixer (for use with Maximizer)			
0.5 to 10 ml/min	none	750 µl	1/4-20 x 1/2" (1.3 cm)
10 to 40 ml/min	5 ml mixer barrel extender	5 ml	1/4-20 x 1-1/2" (3.8 cm)
40 to 80 ml/min	12 ml mixer barrel extender	12 ml	1/4-20 x 3-1/4" (8.2 cm)

To attach the Mixer to the rack:

1. Confirm that the selected mixer and its capacity volume is appropriate for the flow rate. If it is not, refer to the previous chapter for the procedure for changing the mixer capacity.
2. Attach the mixer to a vertical bar using its rod clamp. The mixer should be positioned between the pump and the AVR7-3 inject valve. Attach the mixer to the rack so that the mixer outlet port faces upward.
3. Connect the mixer signal cable (mini-DIN connector) to the connector marked Mixer on the rear of the Maximizer, if available. Otherwise, connect to the Workstation. Refer to Figures 3-4 and 3-5.
4. Plug the unused inlet port using the plug provided.

3.6 DETECTION SYSTEM CONNECTIONS

This section discusses how to connect the UV detector, the Conductivity monitor, the QuadTec detector, and non-Bio-Rad UV detectors.

3.6.1 UV Detector and Conductivity Monitor

Two flow cells are available for use with the UV detector.

- Analytical 5 mm flow cell for high resolution protein chromatography applications and low flow rates. It has a path length of 5 mm for maximum sensitivity and a volume of only 16 μ l. It can be used with flow rates from 0.1 to 10 ml/min.
- Preparative 2 mm flow cell for work not requiring high sensitivity, or when working with high protein concentrations, and for flow rates greater than 10 ml/min. It has a path length of 2 mm and a volume of 30 μ l.

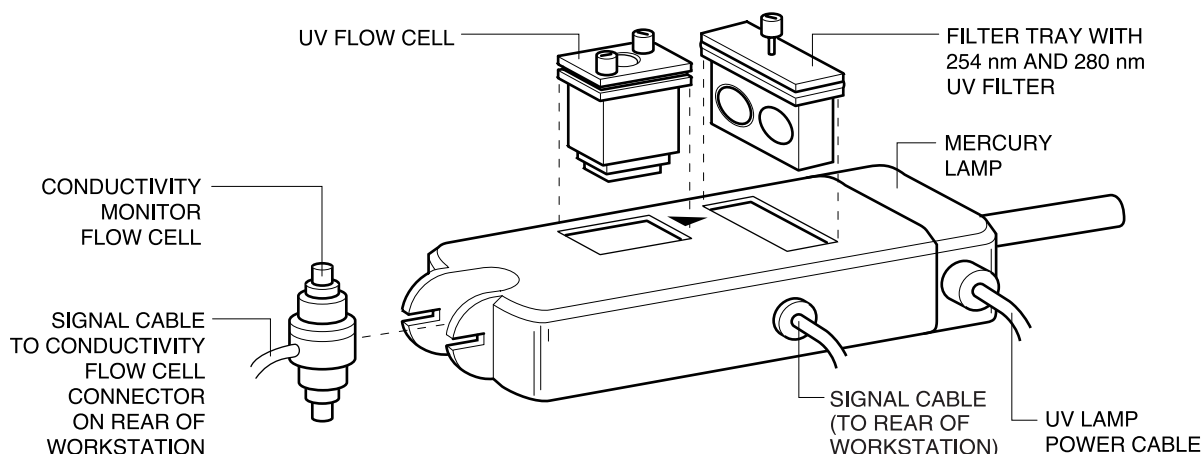


Figure 3-8. UV Detector and Conductivity Monitor

To attach the UV Detector and Conductivity Monitor to the rack:

- Using the information provided above, confirm that the UV flow cell capacity volume is appropriate for the flow rate. Complete discussion of the UV detector and conductivity monitor is provided on pages 2-22 and 2-23, including the procedure for changing the UV flow cell.
- Using the rod clamps attach the UV detector to a vertical or horizontal bar close to the column outlet.
- Connect the power cable (square connector) from the UV detector lamp into the connector marked **UV Lamp** on the rear of the Workstation. Refer to Figures 3-4 and 3-5.
- Connect the UV detector signal cable (mini-DIN connector) to the connector marked **UV Optics** on the rear of the Workstation. Refer to Figures 3-4 and 3-5.
- Connect the Conductivity monitor's combined power and signal cable (mini-DIN connector) to the connector marked **Cond. Flow cell** on the rear of the Maximizer, if available. Otherwise, connect it to the Workstation. Refer to Figures 3-4 and 3-5.
- The Conductivity monitor is designed to be held in the circular notch of the optics bench, but may be placed anywhere in the fluid path.

3.6.2 QuadTec UV/Vis Detector

The QuadTec detector is shipped with a “dummy” flow cell installed. Before operating the detector, you need to install the biocompatible 3mm PEEK flow cell. For complete discussion of the QuadTec detector, including the procedure for installing the flow cell, refer to the QuadTec Instruction Manual.

- **Maximizer:** If the Maximizer is to be used, the QuadTec should be connected to the Maximizer rear panel Com 1 connector using System Cable 25.
- **Workstation:** If the Maximizer is not in use, connect the QuadTec via the Instrument Control Module (ICM), as discussed on page 3-11.

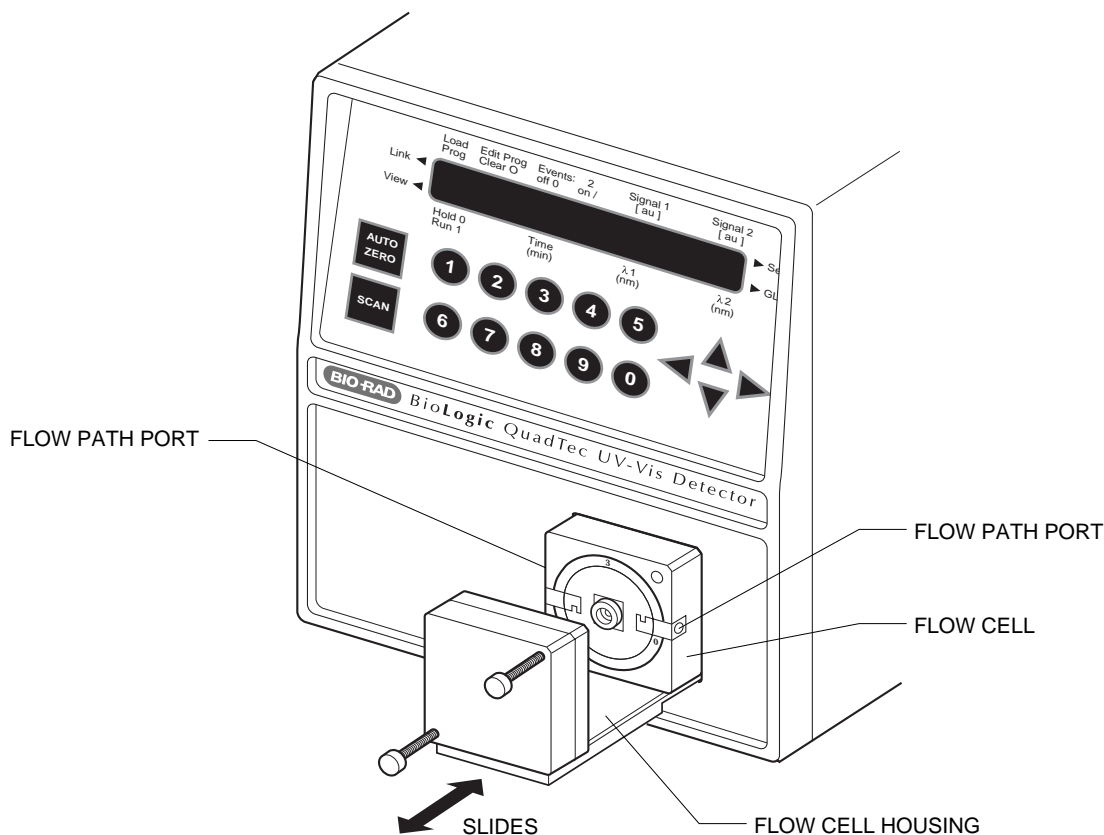


Figure 3-9. QuadTec Detector

The ICM translates the signal from the QuadTec detector and transmits it to the DuoFlow Workstation.



Figure 3-10. Instrument Control Module (ICM)
(front and rear views; for use when the Maximizer is not to be used)

The ICM contains the following:

Front View of ICM Module (required if Maximizer is not in use)

- Address setting: dial should always be set to position 1.
- Instrument bus connector: System Cable 17 connects to the Workstation and the BioFrac fraction collector (if used). (Refer to Figures 3-4 and 3-5.)

Rear View

- Serial Comm connector: System Cable 25 (QuadTec RS232 cable), connects to the QuadTec detector.
- Power connector: System Cable 26, connects to the DuoFlow Workstation DC outlet.

The QuadTec detector is equipped with a universal power supply, which operates with supply voltages from 90 to 260 Volts AC. A manual setting of the supply voltage is not required.

CAUTION! Make sure to use a properly grounded power outlet and the power cable provided with the system.

To connect the QuadTec to the Workstation via the ICM module,

1. Use System Cable 25 to connect from the RS232 connector on the back of the QuadTec to the COMM connector on the ICM module.
2. Use System Cable 26, the ICM power cord, to connect to the back of the Workstation.
3. Use System Cable 17 to connect between an instrument bus connector on the ICM and an available instrument bus connector on the Workstation.
4. If you are using a Bio-Rad device with an instrument bus connector, such as the BioFrac fraction collector, use another System Cable 17 to connect between the instrument bus connectors on the BioFrac and the ICM.

3.6.3 pH Monitor

The pH monitor, available as an option from Bio-Rad, (refer to section 2.5.4 for more information) may be connected to the DuoFlow system in one of two ways:

- To a Workstation connect the Signal Import Module (SIM) included with the pH Monitor. The SIM connects to the DuoFlow Workstation through the bus communication cables (System Cables 17, 18, 19, 21, or 30).
- To a Maximizer, if in use, connect to the rear BNC connector labeled pH.

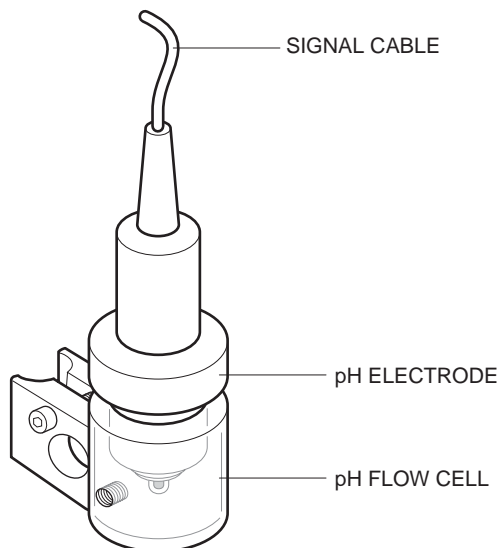


Figure 3-11. pH Monitor

To connect the Bio-Rad pH electrode to the SIM,

1. Connect the output cable from the pH electrode to the connector labeled pH Connector on the SIM.
2. Connect the SIM to the USB Bitbus Communicator by using an Instrument Bus cable (System Cables 17, 18, 19, 21, or 30), as shown below.

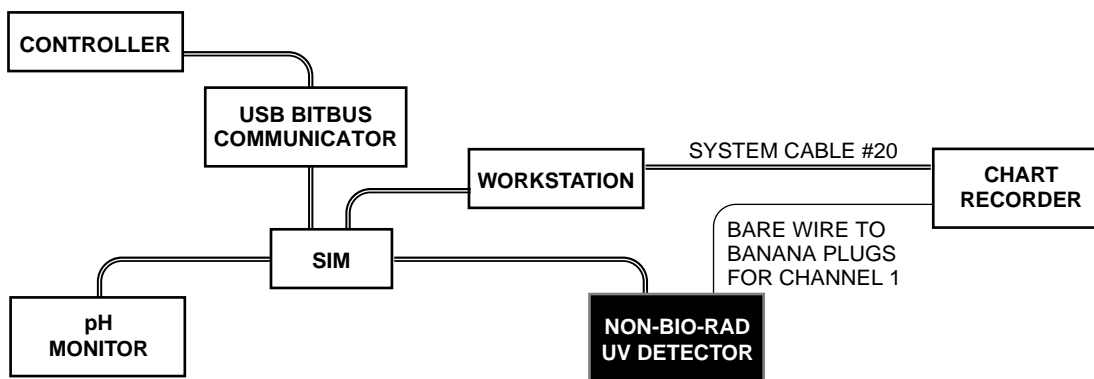


Figure 3-12. SIM Connections

3.6.4 Non-Bio-Rad Detectors

The Signal Import Module (SIM), available from Bio-Rad, allows you to connect a variety of additional devices such as UV, fluorescence, and RI detectors. Before connecting the detector to the SIM, consult the documentation provided with the detector to determine the cable requirements for connecting to the 3-pin connector (+, -, Gnd) on the SIM.

The SIM connects to the DuoFlow Workstation and Controller through the use of the Instrument Bus cables (System Cables 17, 18, 19, 21, or 30), as shown above. Channel 1 signals are sent to the chart recorder by connecting the UV detector to the chart recorder using bare wires to banana plugs. System Cable 20 transmits start/stop, pen up/down, and event mark signals.

3.7 VALVE CONNECTIONS

The DuoFlow system is shipped with an AVR7-3 inject valve. The connection of all valves is similar, as discussed below:

- To connect either the AVR7-3 inject valve or an AVR9-8 stream select valve, mount the valve to a vertical bar on the system rack and connect its cable to any of the connectors labeled Automated Valves 10, 11, or 12 on the rear of the Maximizer, if available. Otherwise, connect it to connectors 4, 5, or 6 on the Workstation.
- To connect an SVT3-2 diverter valve or the SV5-4 buffer select valve, mount the valve to a vertical bar on the system rack and then connect its cable to any of the connectors labeled Solenoid Valves 7, 8, or 9 on the rear of the Maximizer, if available. Otherwise, connect it to connectors 1, 2, or 3 on the Workstation.
- Refer to section 2.6 for more detailed information about each valve.

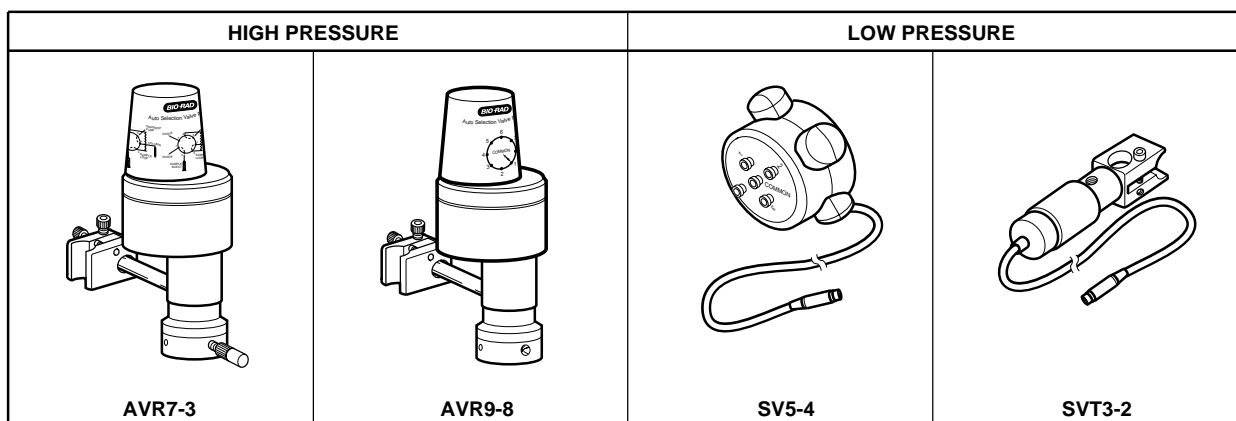


Figure 3-13. DuoFlow Valves

3.8 FRACTION COLLECTOR CONNECTIONS

This section discusses the connections for the following instruments and devices:

- BioFrac fraction collector
- Model 2110 fraction collector
- Model 2128 fraction collector

Refer to section 2.7 for more detailed information about the fraction collectors.

3.8.1 BioFrac Fraction Collector

The BioFrac fraction collector is controlled by the DuoFlow software version 4.0 or greater via the Instrument Bus. The BioFrac fraction collector is connected to the system as discussed below:

1. Connect the USB Bitbus to the Controller as discussed in section 3.2, page 3-3.
2. Use either of the instrument bus connectors on the rear of the fraction collector to connect the BioFrac fraction collector to the Instrument bus.

3.8.2 Model 2110 Fraction Collector

The Model 2110 fraction collector may be connected to the Workstation, as discussed below:

1. Connect the DB-9 connector on System Cable 5 to the Model 2110 fraction collector.
2. Connect the bare wires to the AUX connector on the Workstation as follows: Black wire to pin 5 and white wire to pin 9.

3.8.3 Model 2128 Fraction Collector

The Model 2128 fraction collector is controlled by the DuoFlow Controller via the Instrument Bus. The Model 2128 fraction collector is connected to the system as discussed below:

1. Connect the USB Bitbus to the Controller as discussed in section 3.2.
2. Use either of the instrument bus connectors on the rear of the fraction collector to connect the Model 2128 fraction collector to the Instrument bus.

3.9 PUMP CONNECTIONS

This section discusses the connections for the following instruments and devices:

- Model EP-1 Econo pump
- Econo Gradient Pump (EGP)

3.9.1 Model EP-1 Econo Pump

The EP-1 Econo pump is connected to the system as discussed below:

1. Obtain System Cable 7.
2. Locate the red and blue wires at the wire end of the cable and connect them to the DuoFlow Workstation.
 - a. Connect the red wire to pin 6 of the Aux connector.
 - b. Connect the blue wire to pin 9 of the Aux connector.
 - c. Cut short all other wires and insulate with tape.
3. Plug the 8-pin mini-DIN connector into the Aux connector on the rear panel of the Econo pump.

3.9.2 Econo Gradient Pump (EGP)

Refer to section 2.8.3 page 2-45 for more information. The EGP is connected to the Workstation using an Instrument Bus communication cable (System Cables 17, 18, 19, 21, or 30). Connect its power cable to an available outlet. Be sure to use the appropriate fuse. Refer to the separate documentation for the EGP for fuse specifications.

3.10 MODEL 1327 CHART RECORDER CONNECTIONS

The Model 1327 chart recorder is optional. It may be positioned on the rack shelf or on the bench.

1. Connect System Cable 2 between the Workstation and the recorder as follows:
 - a. The mini-DIN connector is connected to the connector marked "UV Chart" on the rear of the Workstation. Refer to Figures 3-4 and 3-5.
 - b. The DIN connector is connected to the single DIN connector on the side of the chart recorder. This cable provides system control of pen up/down, event marks and paper advance, but chart speed **MUST** be set on the recorder faceplate itself.
2. Conductivity signals are recorded on channel 2 of the recorder using System Cable 4 as follows:
 - a. The mini-DIN connector is connected to the connector marked "Cond. Chart" on the rear of the Workstation. Refer to Figures 3-4 and 3-5.
 - b. The banana plugs are connected to the connectors marked CH 2 on the side of the recorder (red wire to +, black wire to ground |).
3. Set both channel inputs on the recorder to 1V. Set all other switches to their position marked in green. Connect the power adapter to the chart recorder.

3.11 COMPLETING SYSTEM SETUP

Once you have completed the system setup, turn the units around. Connect the USB cable to the USB Bitbus communicator and connect the Instrument Bus cables. Plug in the power cords, and turn on the system. In certain laboratory environments, an uninterruptable power supply (UPS) may be required. Bio-Rad offers both 110 V and 220 V UPS configurations; consult your local Bio-Rad representative.

3.11.1 DuoFlow System Network Connections

The DuoFlow system can be connected to an Ethernet network, allowing you to print reports and export files over the network. Refer to the documentation provided with your PC computer for details on cable connection and software setup. Contact your site administrator to ensure proper communication and access with the existing network.

3.11.2 System Power Up

Power up the system. The following is intended as a checklist of instruments and devices that may be connected to the system. The sequence is not important, although it is best to power on the Controller last.

1. Turn on power to the DuoFlow Workstation.
2. Turn on power to the Maximizer, if in use.
3. Turn on power to the following devices and instruments, if they are available:
 - a. Power up the QuadTec using the power switch on the rear of the detector.
The QuadTec detector goes through a startup routine, self-test, and lamp calibration routine.
 - b. Power up the auxiliary pump. Auxiliary pumps include the EP-1 Econo pump and the Econo Gradient Pump (EGP).
 - c. Power up the fraction collector. Fraction collectors include the BioFrac, the Model 2110, and the Model 2128.
4. Power up the Controller. At this point, the DuoFlow software application may be launched.
5. When the DuoFlow Controller establishes communication with the system, the faceplate in the Manual screen and the status bar at the bottom of the screen show which devices and instruments are connected and communicating with the system. The system is ready for operation.

4.0 SYSTEM PLUMBING

This chapter discusses recommended plumbing practices and provides general guidelines for system setup.

The system will work more efficiently if tubing lengths are as short as possible. Bio-Rad provides precut and 1/4-28 fitted, labeled tubing in the Fittings Kit. The illustration below shows where the tubing is designed to be connected. Discussion of how to create your own tubing can be found in the following section.

Use 1/16" (1.6 mm) OD PEEK tubing and 1/4-28 fittings. Use orange PEEK 0.020" (0.51 mm) ID tubing for the F10 pumps, and green PEEK 0.030" (0.76 mm) ID tubing for the F40 pumps. Premade tubing kits are provided with your Fittings kit.

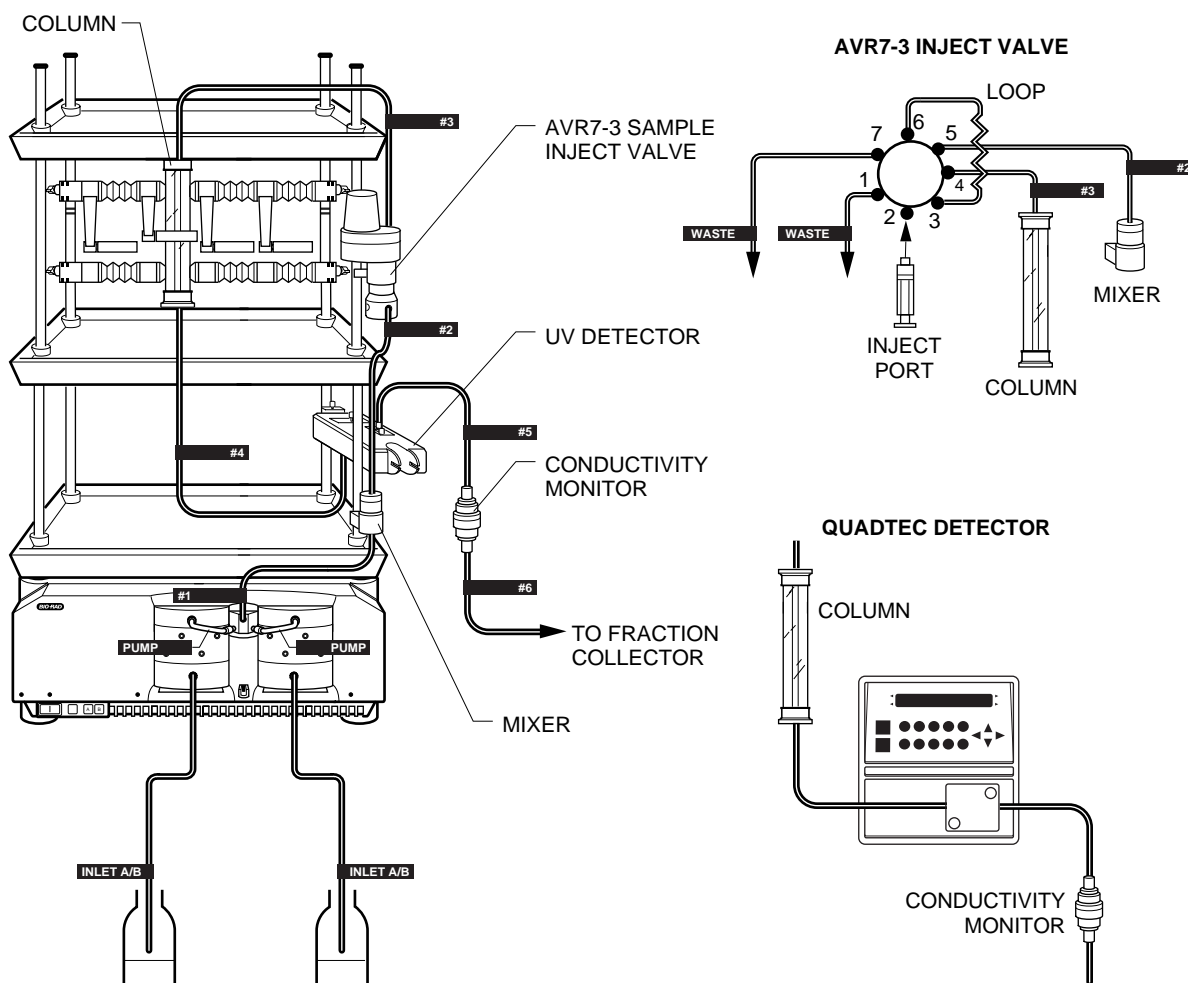


Figure 4-1. System Plumbing using Tubing Kit from Fittings Kit

4.1 GENERAL GUIDELINES FOR CREATING YOUR OWN TUBING CONNECTIONS

The DuoFlow system uses three types of tubing. Use the following table to select the appropriate tubing.

The Fittings Kit comes with fittings, tubing, and an F10 Tubing Kit, with the necessary tubing cut and fitted for each system connection (see Figure 4-1). Complete description of each of these items is provided with the Fittings kit.

Table 4-1.
Tubing Guidelines

Use	Tubing Dimensions	Tubing Material	Fittings	Vol/cm
Pre-pump* (Workstation and Aux pumps)	1/8" OD x 0.062" ID (3.2 mm OD x 1.6 mm ID)	clear, Teflon	Super flangeless fittings for 1/8" (3.2 mm) OD tubing	20 µl
Post-pump**	1/16 OD x 0.02" ID (1.6 mm OD x 0.5 mm ID)	clear, Tefzel	Super flangeless fittings for 1/16" (1.6 mm) OD tubing	2 µl
Post-pump** with F10 pumphead	1/16" OD x 0.020" ID (1.6 mm OD x 0.5 mm ID)	orange, PEEK	1/4-28 Super flangeless fittings for 1/16" (1.6 mm) OD tubing	2 µl
Post-pump** with F40 pumphead	1/16" OD x 0.030" ID (1.6 mm OD x 0.76 mm ID)	green, PEEK	1/4-28 Super flangeless fittings for 1/16" (1.6 mm) OD tubing	4.5 µl

* Pre-pump refers to all tubing leading up to pump inlet port. Pumps include Workstation pumps, EP-1 pump, and Econo Gradient Pump. Maximizer uses pre-pump tubing.

**Post-pump refers to all tubing following the pump outlet port.

When plumbing the system, be sure to keep tubing lengths to a minimum. All fittings should be finger-tight. Do not over-tighten, as you risk damaging the connection.

Ferrule Installation: 1/8" (3.2 mm) OD and 1/16" (1.6 mm) OD tubing

1. Cut the tubing with the tubing cutter provided in the Fittings Kit. This will give a flat, clean cut to the tubing; this is important in making a fitting that does not leak.

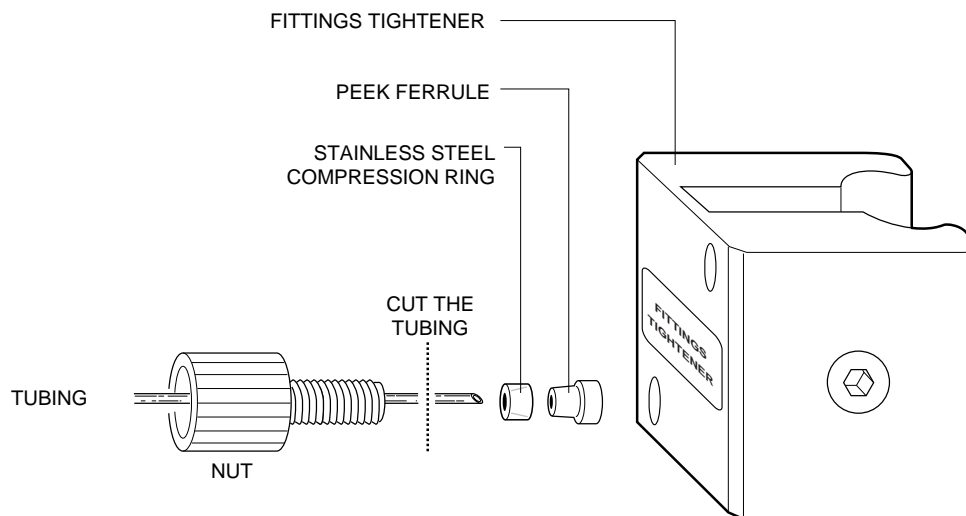


Figure 4-2. Making 1/4-28 flat bottom fittings

2. Slide the nut, stainless steel compression ring, and the ferrule, in that order, onto the tubing as shown in figure 4-2. The flattened end of the compression ring should face towards the nut with the tapered end facing the tapered end of the brown ferrule.
The stainless steel lock ring is *not* in the fluid path, so biocompatibility is maintained.
3. Allow the tubing to extend slightly beyond the end of the ferrule.
4. Place the fitting and tubing into the green fittings tightener. Do not allow the tubing to slip out of the ferrule.
5. Tighten with your fingers to seat the ferrule onto the tubing, but do not over-tighten. The ferrule should be flush with the tubing.
6. Once the fitting is made, the compression ring and ferrule should adhere to the end of the tubing while the nut will be moveable.

4.2 PLUMBING A DUOFLOW SYSTEM

This section discusses how to create your own plumbing arrangement of a DuoFlow system. The plumbing for each of the DuoFlow valves is discussed later in the chapter.

The Fittings Kit includes an F10 tubing kit that includes all tubing required for a basic installation. Refer to figure 4-1.

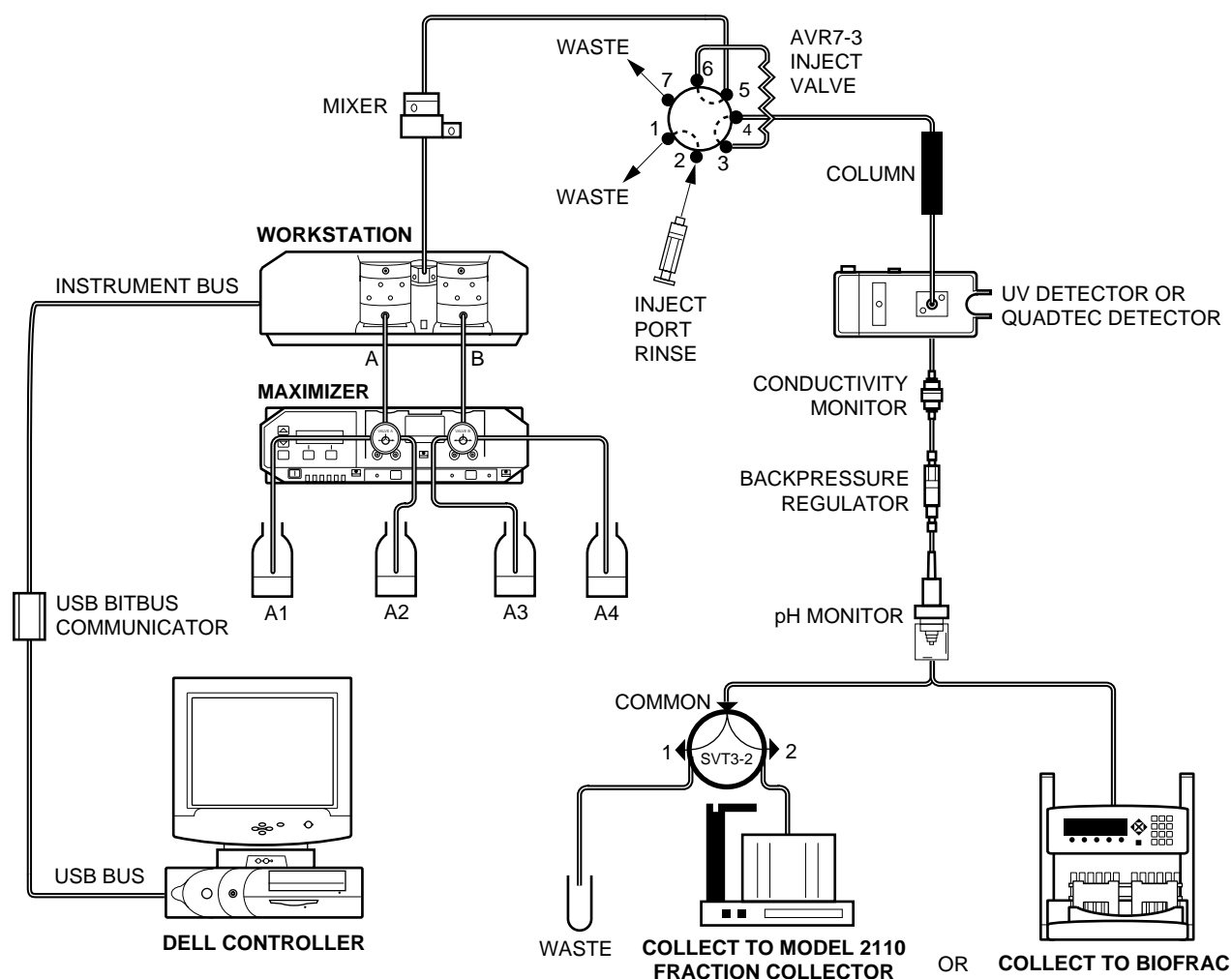


Figure 4-3. System Plumbing with Maximizer

1. Plumbing the Maximizer.

The Maximizer Tubing Kit provides colored FEP Teflon 1/8" OD, 0.062" ID, prefitted, tubing lengths.

Plumbing the Buffer Reservoirs to the Inlets on Maximizer Valves A and B

- a. From the Maximizer Tubing Kit, identify the following:
 - The red tubing labeled Inlet A1 connects the buffer container to the Maximizer valve port A1.
 - The blue tubing labeled Inlet A2 connects the buffer container to the Maximizer valve port A2.
 - The yellow tubing labeled Inlet B1 connects the buffer container to the Maximizer valve port B1.
 - The green tubing labeled Inlet B2 connects the buffer container to the Maximizer valve port B2.For complete discussion of Maximizer tubing installation, refer to the Maximizer Tubing Kit diagram.
- b. Screw the tubing into the inlet connectors on the sides of the valves. Ensure a firm connection but do not over-tighten.

Plumbing the Maximizer Valve Outlets to the Workstation Pump Inlets

- c. Connect the two preformed fittings provided between the Maximizer valve ports and the Workstation pump inlet ports. Connect to the Workstation first.
- d. Because the tubing is rigid, you will need to lower the Maximizer valves in order to connect the tubing. This requires loosening the two screws at the base of each valve so that you can tilt the valve downward. Refer to the illustration below.
- e. Insert the tubing fitting into the Maximizer outlet port and screw in the fitting.

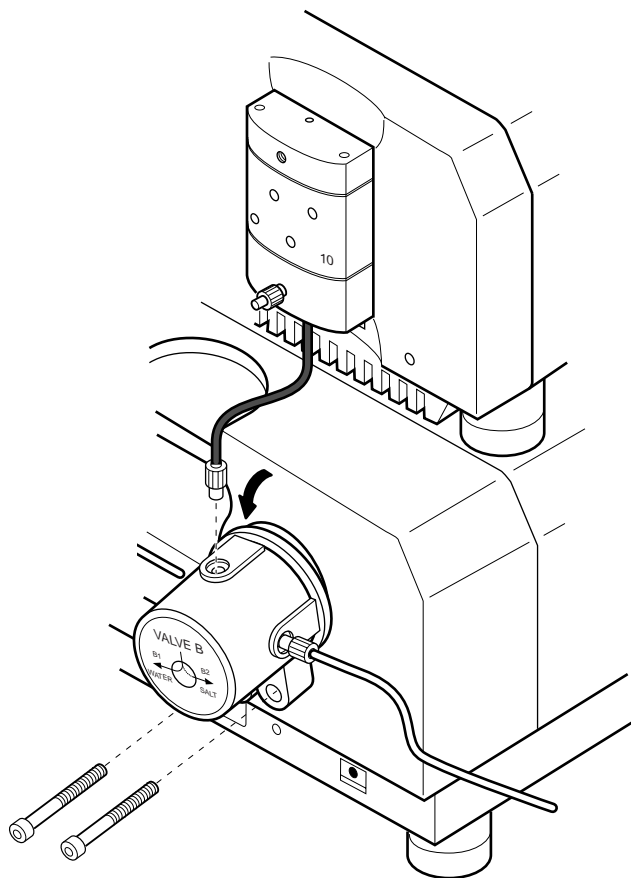


Figure 4-4. Maximizer Plumbing

2. Plumbing the Workstation pump Inlets.

- Workstation pump inlets, attach two fittings to 1/8" (3.2 mm) OD Teflon tubing as described earlier. (See Section 4.1, General Guidelines for Creating Your Own Tubing.)

To connect the SV5-4 buffer select valve or the SVT3-2 valve before the pump inlet, 1/8" (3.2 mm) OD Teflon tubing is used.

- Screw the tubing into the inlet connectors on the bottom of the Workstation pump housing. Ensure a firm connection but do not over-tighten.
- Place opposite end of tubing in solution bottles A and B. Refer to figure 4-1. If an SV5-4 is in use, attach the pump inlet tube to its common port. Refer to section 2.6.3, page 2-31.

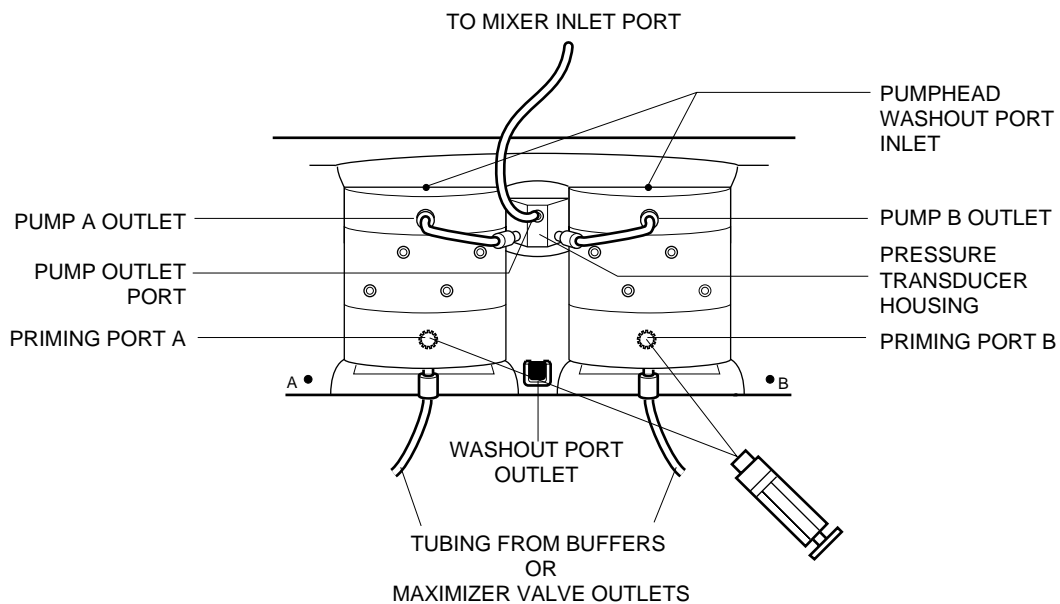


Figure 4-5. Plumbing Connections to the Workstation Pump

3. Plumbing from the Workstation pumpheads to the Transducer (Figure 4-3).

- Make two fittings using 1/16" (1.6 mm) OD orange PEEK tubing and 1/4-28 fittings, as described in section 4.1.
- Connect the tubing to each pumphead outlet port and to each transducer inlet port. Ensure a firm connection but do not over-tighten.

4. Plumbing from the Transducer to the Mixer (Figures 4-2 and 4-3).

- Make a fitting using 1/16" (1.6 mm) OD orange PEEK tubing and 1/4-28 fittings.
- Connect the tubing to the transducer outlet port and to either of the inlet ports on the mixer. Plug the unused mixer inlet port using the plug provided for this purpose. Ensure a firm connection but do not over-tighten.

5. Plumbing from the Mixer to the AVR7-3 Inject Valve.

- Cut a suitable length of 1/16" (1.6 mm) OD orange PEEK tubing that will reach from the mixer outlet port to port 5 of the inject valve. Attach 1/4-28 fittings to each end.
- Connect the tubing to the mixer outlet port and to port 5 on the inject valve. Ensure a firm connection but do not over-tighten.

6. Plumbing the AVR7-3 Inject Valve.

- The inject port assembly and needle are included with each AVR7-3 inject valve. Screw this assembly into port 2 of the valve until it is secure.
- Connect the sample loop to ports 3 and 6.
- Plumb the inject valve according to Figure 4-6. Make a fitting using 1/16" (1.6 mm) OD Tefzel tubing and 1/4-28 fittings for waste lines 1 and 7.

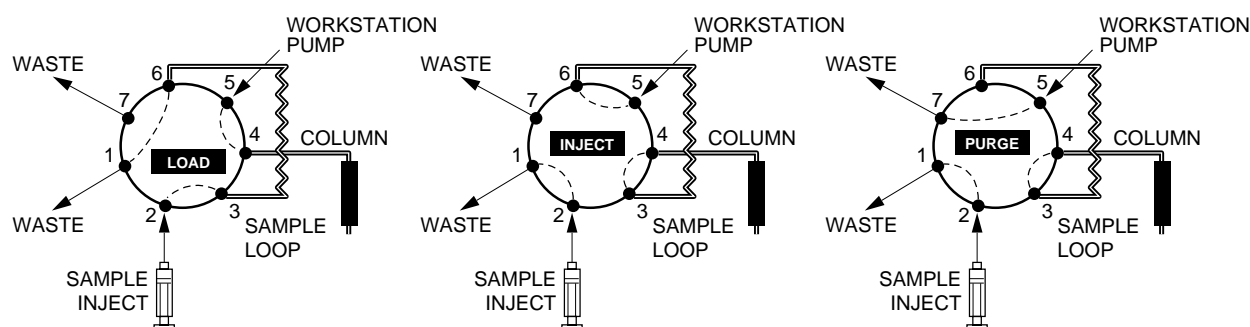
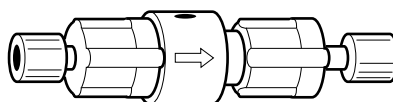


Figure 4-6. Inject Valve Plumbing for an AVR7-3

7. Plumbing the UV Detector and Conductivity Monitor.

- Connect a piece of 1/16" (1.6 mm) OD orange PEEK tubing between the column outlet and the bottom inlet of the UV detector flow cell using 1/4-28 fittings. If you are using the QuadTec detector, connect it to the flow cell which is bidirectional, using 10-32 fittings provided with the QuadTec.
- Cut approximately 8 cm of 1/16" (1.6 mm) OD orange PEEK tubing and attach 1/4-28 fittings. Connect one end into the top of the UV detector flow cell. Connect the other end into the Conductivity monitor flow cell which is bi-directional. If you are using a QuadTec detector, connect tubing from its flow cell outlet using a 10-32 fitting to either port of the Conductivity monitor using 1/4-28 fittings.
- Place the Conductivity flow cell into the notch of the optics bench. This is a gentle push-fit. There is a tag (with a number) attached to the conductivity cable. This number is the flow cell constant and must be entered in the software before beginning a run. Refer to Table 5-6, page 5-10 Utilities drop-down Menu: Conductivity Flow Cell Constant Calibration.
- For flow rates below 10 ml/min, insert the 40 psi backpressure regulator after the conductivity monitor. Plumb the backpressure regulator following the direction of the arrow.



FLOW DIRECTION ➡

Figure 4-7. Backpressure Regulator

The backpressure regulator is required to help eliminate bubbles from becoming trapped in the detector flow cell.

When using low pressure columns such as an Econo-Pac® cartridge or Econo column, plumb the 40 psi backpressure regulator at the Workstation pump outlet. This aids in seating the check valves, preventing permanent damage to the cartridge or column.

Refer to section 2.9.5, page 2-52 for more information.

8. Connection to a Fraction Collector.

Connection to a BioFrac Fraction Collector

- a. Make a fitting using 1/16" (1.6 mm) OD orange PEEK tubing and 1/4-28 fittings.
- b. Connect the fitting between the Conductivity flow cell (or the backpressure regulator or pH monitor flow cell, if they are installed) and the BioFrac. (For complete instructions, refer to the documentation for the BioFrac fraction collector.)

Connection to a Model 2128 Fraction Collector

- a. Make a fitting using 1/16" (1.6 mm) OD orange PEEK tubing and 1/4-28 fittings.
Assure that the tubing length allows unrestricted movement of the fraction collector arm.
- b. Connect the fitting between the Conductivity flow cell (or the backpressure regulator or pH monitor flow cell, if they are installed) and the drop-head of the Model 2128 or the Model 2128 diverter valve. (For complete instructions, refer to the documentation for the Model 2128 fraction collector.)

Connection to a Model 2110 Fraction Collector and a SVT3-2 Fraction Collector Diverter valve.

- a. Connect a piece of 1/16" OD orange PEEK tubing between the Conductivity flow cell (or the backpressure regulator, if that was installed) and the SVT3-2 valve common port (labeled "Common") using the 1/4-28 fittings.
- b. Connect a piece of 1/16" OD Tefzel tubing from port 1 of the SVT3-2 valve (see Figure 4-3) for use as a waste line.
- c. Connect a piece of 1/16" OD Tefzel tubing from port 2 of the SVT3-2 valve (see Figure 4-3) to the Model 2110 fraction collector drop-head. This tubing sits inside the drop-head, and no fittings are required.

4.3 PRIMING THE SYSTEM

Before a method can be run, the system must be purged of air. Prior to running aqueous solutions through the system, pump 200 ml of methanol (MeOH) through each pumphead at 5 ml/min with the 40 psi backpressure device placed in line at the pump outlet. Once this is done, prime the remainder of the system with water.

1. Priming the Workstation pump.

- If you are not using a Maximizer, immerse the Workstation pump A and B inlet lines in a container of HPLC grade (filtered, degassed) or other high quality water.
- Place the 10 ml luer syringe (supplied with the fittings kit) on the priming port of pumphead A.
- Turn the priming port counter-clockwise to open the seal and gently withdraw the syringe plunger to draw water into the pumphead.
- Repeat this operation until no air bubbles are visible in the inlet tubing.
- Remove the syringe and tighten the priming port by turning it clockwise.
- Repeat this priming procedure for pumphead B. See Section 11.3.1 for additional priming procedures.

2. Priming the Maximizer valves.

- Immerse the inlet lines in a container of HPLC grade (filtered, degassed) or other high quality water.
- Using the button below the Valve A, select port A1.
- With the AVR7-3 inject valve in purge mode, place a 10 ml luer syringe (supplied with the fittings kit) into port 3 of the AVR7-3 valve and draw air out of the system.
- Repeat the procedure for each Maximizer valve position.

3. Moving the AVR7-3 Inject Valve to Purge Position.

- Go to the Manual screen displayed by the system software.
If you connected an AVR7-3 valve into port 4 on the Workstation, you will see a valve box designated "AVR7-3 at port 4." The three radio buttons of this box correspond to valve positions as follows: L = Load position, I = Inject position, P = Purge position.
- To move the AVR7-3 valve to Purge position, click button P.

The default position at power up and at the end of a programmed method for the AVR7-3 is position L, and for all other automated valves is position 1 unless changed in the Edit User Preferences window, available from the Options menu.

4. Purging the Workstation pump.

With the AVR7-3 valve in position P (Purge):

- Press the Purge buttons A and B on the front of the Workstation. The Workstation pump will run and the indicator lights will flash green. The default purge flow rate for the F10 pumps is 10ml/min; that for the F40 pumps is 40 ml/min. (This may be changed using the Manual Setup window, available from the Options menu.)
- Run both pumps for about 2 minutes and then press the Purge buttons again to stop the pump.

5. Flushing the system through to the fraction collector.

With the Workstation pump stopped, move the inject valve back to position I (Inject) by clicking the radio button I (AVR7-3) on the Manual screen.

Using the Manual screen, set the pump flow rate at 1.0 ml/min and start the pump. Water will now flow through the sample loop, the UV and Conductivity flow cells, and ultimately to the fraction collector.

If the system pressure variations exceed $\pm 10\%$, refer to section 11.3.1, Priming the Pump and Removing Trapped Air Bubbles.

5.0 INTRODUCTION TO THE SYSTEM SOFTWARE

The BioLogic DuoFlow system software is based on Microsoft Windows® 2000 for PC-compatible computers. This chapter discusses the DuoFlow system software version 4.0.

5.1 SYSTEM INTERFACE

The Manual screen (Figure 5-1) is the first screen displayed when you turn on the system. **All menus and tool bar functions will be grayed out and inaccessible until you select, or enter a User name in the Browser, refer to chapter 6, page 6-1.** This screen, like all DuoFlow screens, is grouped into the system menus, the control window, and the status bar. The use of the Control window functions is discussed in Chapters 6 and 7 and in the separate documentation for the Econo Gradient Pump (EGP) and the QuadTec UV/VIS detector.

- Browser screen: Chapter 6
- Manual screen: Chapter 7, section 7.1
- Setup screen: Chapter 7, section 7.2
- Protocol screen: Chapter 7, section 7.3
- Run screen: Chapter 7, section 7.4
- Post Run screen: Chapter 7, section 7.5

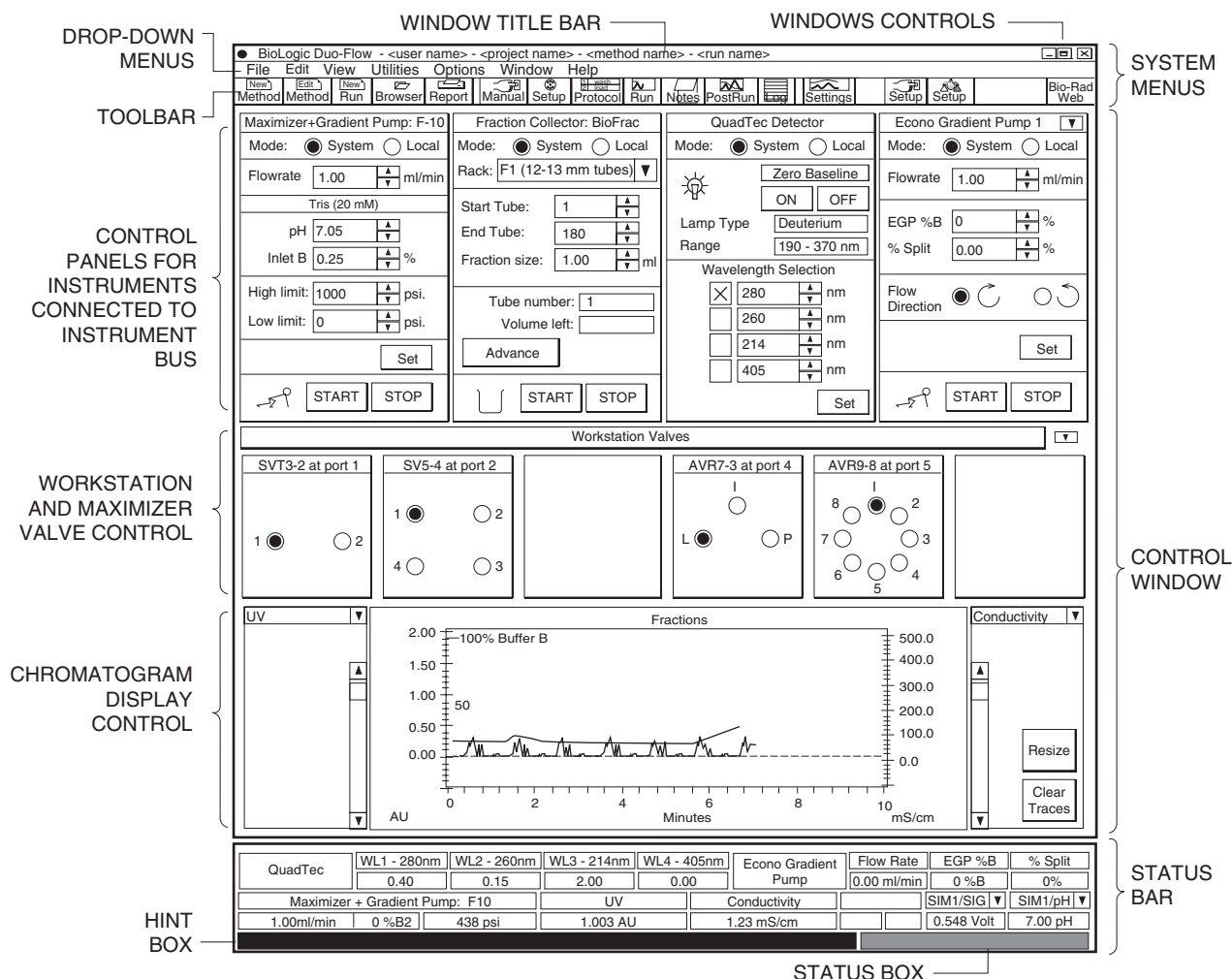


Figure 5-1. Layout of the Screen Display, showing the Manual Screen

The DuoFlow system is controlled and monitored using the following:





- **Toolbar:** The toolbar is the primary navigation tool for the system software.
- **Drop-down menus:** The drop-down menus provide access to advanced functions. Some functions found on the toolbar are duplicated in the drop-down menus.
- **Status bar:** The status bar provides realtime information about active instruments and devices connected to the system, for example system backpressure and detector AU readings.

Items shown in gray are not currently active.

5.2 STANDARD MOUSE AND KEYBOARD FUNCTIONS

The DuoFlow system is supplied with a Dell PC computer. The left mouse button is used with system software, except as noted.

Table 5-1. Special Function Keys

Special Function Keys	Description
	Hold until Keypress: Used during a sample run to continue a method (i.e., satisfy the Hold) when the method includes a “Hold until Keypress” step.
	Help: Displays the Help menu for the currently displayed screen.
	Esc: Functions as an alternative to the Cancel button in a dialog box.
	Alt: Some system commands can be executed either by selecting them from a drop-down menu or by holding down the Alt key and then pressing the appropriate character key.

5.3 SYSTEM MENUS

The system menus consist of both drop-down menus and the toolbar. In some cases, identical functions are found in both areas. Advanced features are located only in the system drop-down menu.

5.3.1 Toolbar Buttons

The function of each toolbar button defined in Table 5-2 is duplicated in the File and View drop-down menus..

Table 5-2.
Toolbar Buttons








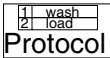




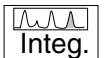




Button	Description
	Initiates the process of writing a new method. The new method will be saved into the open User and Project folder selected in the Browser and listed in the window title bar.
	Initiates the process of editing a method when an open method has runs associated with it. A new method is created based on edits made to the open method. You will be prompted for a new method name or the methods will automatically be numbered consecutively.
	Initiates a new run for the open method. You will be prompted for a new run name prior to launching the run, or the runs will automatically be numbered consecutively.
	Opens the database of existing users, projects, methods, and runs for creating new users and projects. Refer to Chapter 6, Browser Screen. All menus and tool bar functions will be grayed out and inaccessible until you select, or enter a User name in the Browser, refer to chapter 6, page 6-1.
	Allows you to determine when to print and what is to be printed in the report for a run.
	Displays the Manual screen, enabling manual input to instruments and devices in the system. The Manual screen chromatogram is for baseline tracking and can be viewed only.
	Displays the instrument Setup screen for specifying the components required for your method. From the Setup screen you can add a descriptive name to the valve positions and buffers. This screen is automatically displayed after the New Method button is selected.

Table 5-2. Toolbar Buttons (continued)

Button	Description
 Protocol	Displays the Protocol screen for creating and editing a method.
 Run	Displays the Run screen, from which you can initiate a run of the currently open method. You will be prompted for a new run name prior to launching the run, or the runs will automatically be numbered consecutively.
 Notes	Displays the Notes screen that allows you to enter additional information about your method and/or run. Notes are always editable, even after a run is executed.
 PostRun	PostRun allows you to customize your chromatogram prior to printing.
 Log	Records all events during a run in the Log. This information cannot be edited. This button is replaced when the EZLogic integration software is installed. In this case, select Run Log from the View drop-down menu, to view the log.
 Integ.	EZLogic Integration software appears when the EZLogic integration software is installed; this button replaces the Log button.
 Settings	Enables the selection of up to 8 instrument traces to display on screen and on the printed report. The trace options are: UV detector, Conductivity monitor, pH, system backpressure, theoretical %B concentration, four QuadTec wavelengths, and detector traces acquired via the System Interface Module (SIM).
 Setup	Available only in the Manual screen. Displays a screen that enables the following inputs: <ul style="list-style-type: none"> Econo Gradient Pump (EGP) split time period when the EGP is to be used. QuadTec UV/VIS detector time constant when the QuadTec detector is to be used. Pump purge rate. Signal Import Module (SIM) signal parameters.
 Setup	Available only when the Maximizer is used with the system. This allows you to switch between buffer blending and non-buffer blending modes. In buffer blending mode, a window listing buffer systems available for use is displayed.
 Bio-Rad Web	If your controller has internet access, selecting this button takes you to the Bio-Rad web page.

5.3.2 Drop-down Menus

The drop-down menus are discussed in the following tables. **All menus and tool bar functions will be grayed out and inaccessible until you select, or enter a User name in the Browser, refer to chapter 6, page 6-1.**

Table 5-3. File Drop-down Menu

● BioLogic Duo-Flow - <user name> - <project name> - <method name>	
File	Edit View Utilities Options Window Help
New Method	
Copy and Edit Method	
New Run	
Open...	
Close	
Select User and Project	
Change Name, Author...	
Save setup ...	
Load setup...	
Print Report...	
Data management...	
Export Data...	
Export Chromatogram Image...	
Integration...	
Exit	
1 Method 1-	
2 Method 2-	
3 Method 3-	
4 Method 4-	

The File menu is always displayed. It consists of the following:

- **New Method:** Initiates the process of writing a new method. The new method will be saved into the open User and Project directories.
 - **Copy and Edit Method:** Allows you to use an existing method as a template for a new method. A copy of the method is made, and you will be prompted for a new method name or the methods will automatically be numbered consecutively.
 - **New Run:** Initiates a run for the open method. You will be prompted for a new run name prior to launching the run or the methods will automatically be numbered consecutively.
 - **Open:** Displays the browser. Refer to Chapter 6, Browser Screen.
 - **Close:** Closes the current method and/or run.
 - **Select User and Project...:** Allows you to select the default user and project of the system when new methods are created.
 - **Change Name, Author...:** Allows you to change the method/run name, author, and description. This function is inactive once a run has been initiated.
 - **Save setup...:** Allows you to save and name different instrument setups configured in the Setup screen and to set one as the default.
 - **Load setup...:** Recalls and loads the selected setup.
-

Table 5-3. File Drop-down Menu (continued)

-
- **Print Report:** Allows you to print a report of the currently open method, including its setup and run data, run results, and the run log report.
 - **Data Management:** Displays the Browser screen, from which you can copy and move run data. Refer to Chapter 6, Browser Screen.
 - **Export Data:** This feature establishes data export parameters, and is available from the PostRun screen.
 - **Export Chromatogram Image:** This feature exports a chromatogram image in a Windows Meta File (.WMF) format. This feature is available in the PostRun screen.
 - **Integration:** Launches the Bio-Rad EZLogic integration software. Contact Bio-Rad Technical Support at 1-800-4-BIORAD in the USA or your local Bio-Rad representative for more information on EZLogic.
 - **Exit:** Exits the BioLogic system software and returns to the Windows desktop.
 - **1 through 4:** Display the last four user names and methods
-

Table 5-4. Edit Drop-down Menu

Edit menu: Setup Screen

● BioLogic Duo-Flow - <user name> - <project name> - <method name>						
File	Edit	View	Utilities	Options	Window	Help
	Edit...					
	Delete					
	Select All					

Edit menu: Protocol Screen

● BioLogic Duo-Flow - <user name> - <project name> - <method name>						
File	Edit	View	Utilities	Options	Window	Help
	Edit...					
	Cut					
	Copy					
	Paste					
	Delete					
	Select All					

Edit menu: Run Screen

● BioLogic Duo-Flow - <user name> - <project name> - <method name>						
File	Edit	View	Utilities	Options	Window	Help
	Full view in Run Chromatogram					
	Multiple runs...					
	Start run					
	Pause buffer pump					
	Hold gradient					

Edit menu: Post Run Screen

● BioLogic Duo-Flow - <user name> - <project name> - <method name>						
File	Edit	View	Utilities	Options	Window	Help
	Edit Activity Trace ...					
	Tag and Trace Options ...					
	Full view in zoom Chromatogram					
	Print zoom Chromatogram					
	Trace Legend					
	Copy zoom Chromatogram to clipboard					
	Delete selected tag					
	Delete all tags					

Note: The Edit menu is not available in the Manual or Browser screens.

The contents of the Edit menu depends upon the displayed screen as indicated above. In most instances, the item in the drop-down menu also appears in the system toolbar; exceptions are noted below.

Setup Screen

- **Edit...:** Allows you to edit the selected device in Setup.
- **Delete:** Deletes the currently highlighted device in Setup. To delete all devices, first select Select All, as described below.
- **Select All:** Highlights all devices in Setup. (Not available from the toolbar.)

Protocol Screen

- **Edit...:** Displays the Edit window for the step selected in the protocol.
- **Cut:** Cuts (deletes) the currently highlighted step from the protocol. A cut step may be pasted elsewhere.
- **Copy:** Copies the currently highlighted step so that it can be pasted elsewhere in the protocol.
- **Paste:** Pastes the cut or copied step into the protocol.
- **Delete:** Deletes the currently highlighted step from the protocol.
- **Select All:** Highlights all protocol steps. Cut, Copy, and Delete then act on all steps. To remove the highlighting from individual steps, hold down the Ctrl key and click the mouse over the desired step. (Not available from the toolbar.)

Run Screen

- **Full view in Run Chromatogram:** Zooms out to show the full view for the run.
- **Multiple runs...:** Specifies the number of times the method is to run.

Table 5-4. Edit Drop-down Menu (continued)

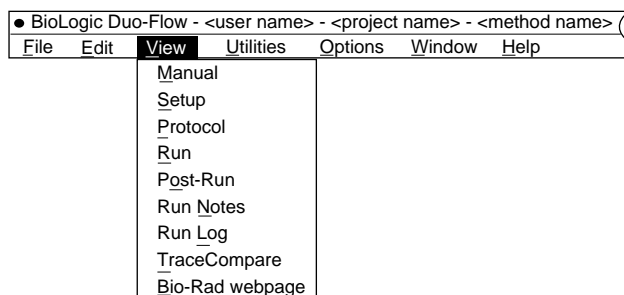
Run Screen (continued)

- **Start run:** Starts the run.
- **Pause buffer pump:** Pauses progression of method's protocol. Stops gradient pumps and the method time (volume) does not advance. From Pause, you can Abort, Continue, or Edit-During-Run.
- **Hold gradient:** Holds the current %B gradient pump conditions and halts the advance of the method's protocol (including fraction collection). The method time (volume) does not advance. From Hold, you can Abort, Pause, or Continue.

Post Run Screen

- **Edit Activity Trace....:** Allows you to input post run sample activity data obtained off-line, refer to page 7-33.)
 - **Tag and Trace Options....:** Displays the Post Run Tags window that allows you to specify which traces are to be displayed, and name peaks. Also available from the **Tag** button in the Toolbar, refer to page 7-32.
 - **Full View in zoom chromatogram:** Allows you to view the complete chromatogram. Also available from the **Full View** button in the system toolbar.
 - **Print zoom chromatogram:** Allows you to print the complete chromatogram. Also available from the **Print** button in the toolbar.
 - **Copy zoom chromatogram to clipboard:** Copies to clipboard the complete chromatogram. From the clipboard, it can then be copied into other applications. Not available from the toolbar.
 - **Delete selected tag:** To delete a tag, highlight the selected tag before using this function. Also available from the **Del. Tag** button in the toolbar.
 - **Delete all tags:** Deletes all tags in the chromatogram. Not available from the toolbar.
 - **Trace Legend:** Displays different line formats for each of the chromatogram traces. This is useful for distinguishing traces when printing to a black and white printer.
-

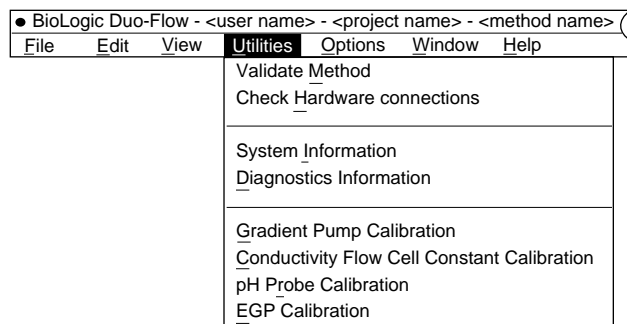
Table 5-5. View Drop-down Menu



The contents of View menu remain the same in each displayed screen.

- **Manual:** Displays the Manual screen for individual control of installed instruments and devices in the system.
 - **Setup:** Displays the Setup screen, which allows you to specify the components required for your method.
 - **Protocol:** Displays the Protocol screen for creating and editing chromatography steps in the method.
 - **Run:** Displays the Run screen, from which you can initiate a sample run of the open method.
 - **Post Run:** When a run is completed, select Post Run to annotate the chromatogram, for example, to apply tags to UV, Conductivity, and/or %B values.
 - **Run Notes:** To keep notes regarding the run, select Run Notes and enter them into the Notes screen. There are fields for description of the sample, the column, the operator, the buffer(s), flow rates, gradients, chart speed, fraction size, and general text entry of variable length. These notes are printed with the report.
 - **Run Log:** Displays a log of all events occurring during the run. This information cannot be edited. The Run Log may be disabled by selecting Edit User Preferences from the Option drop-down menu. It is recommended, however, that the Run Log be active for assistance in troubleshooting.
Note : If the Bio-Rad EzLogic Integration software option is installed, the Log toolbar button is replaced by the Integ toolbar button. To display the Log window, select Run Log from the View drop-down menu.
 - **TraceCompare:** Allows you to compare the trace results from different runs.
 - **Bio-Rad webpage:** If your Controller has internet access, you can use this button to access the Bio-Rad web page.
-

Table 5-6. Utilities Drop-down Menu



The Utilities menu selections relate to system options, and remain the same in each displayed screen. The Utilities menu consists of the following:

- **Validate Method:** Verifies that the devices required by the method Protocol have been defined or selected in the Setup screen. Validation is automatically run at the start of a new run.
- **Check Hardware connections:** Checks that all devices listed in the Setup screen are electrically connected to the system. The mixer is always assumed to be connected, so it does not appear in the Setup screen and its connection is not checked.
- **System Information:** Displays the current system configuration, including each instrument in the system and its firmware version number, the Windows® version number, available hard disk space, and the number of methods and runs in the database.
- **Diagnostics Information:** Displays information for service diagnostic purposes only.
- **Gradient Pump Calibration:** Available only from the Manual screen, and it is typically used only after servicing of the pump. It allows the user to calibrate the gradient pump flow rate and zero the system pressure gauge.
Note: You must exit the Calibration screen in order for the system to accept the calibration values.
- **Conductivity Flow Cell Constant Calibration:** Allows the user to calibrate the conductivity flow cell. The conductivity flow cell constant is printed on a tag attached to the flow cell cable. When a new conductivity flow cell is installed, use this value to enter the flow cell constant.
Note: You must exit the Calibration screen in order for the system to accept the calibration values.
- **pH Probe Calibration:** This utility allows the user to calibrate the pH monitor. It is typically used at the start of each day's use of the system.
Note: You must exit the Calibration screen in order for the system to accept the calibration values.
- **EGP Calibration:** This is informational only. It reminds the user to calibrate the pump through the pump software. Refer to the EGP Instruction Manual.

Table 5-7. Options Drop-down Menu

Options menu: Manual Screen		Options menu: Browser, Setup, Protocol, Post-Run Screen	
<div> <div>● BioLogic Duo-Flow - <user name> - <project name> - <method></div> <div>File Edit View Utilities Options Window Help</div> <div> Edit User Preferences Chromatogram Settings Status lines Browser Settings Manual Setup Buffer Blender Setup </div> </div>		<div> <div>● BioLogic Duo-Flow - <user name> - <project name> - <method></div> <div>File Edit View Utilities Options Window Help</div> <div> Edit User Preferences Chromatogram Settings Status lines Browser Settings * Use Time (min) * Use Volume (ml) </div> </div>	
		* Time and Volume are displayed	

The contents of the Options menu depends up the displayed screen as indicated above. These selections are to change screen options.

- **Edit User Preferences:** Lets you specify the following:
 - Run Options:** Allows you to elect to have the protocol steps listed in the Run Log and if the valves automatically return to position 1 at the end of a run.
 - Protocol Editor Mode:** This determines whether the protocol is to be based on time or volume.
- **Chromatogram settings:** Allows you to select which instrument traces will be visible on the chromatograms. Up to 8 instrument traces are selectable from among the following: standard UV detector, Conductivity monitor, pH, system backpressure, theoretical %B concentration, four QuadTec wavelengths, and detector traces acquired via the Signal Import Module (SIM). For the Manual screen, the X-axis (time) and the Y-axis (AU) ranges can be set. For the Run and Post Run screens, only the Y-axis range can be set.
- **Status Lines:** Allows the status information at the bottom of the screen to be toggled on or off.
- **Browser settings:** This enables you to select a heirarchy of how the Browser is to display users/projects/methods/ runs. For example, select Projects to display a top level folder called "Projects" which contains all projects in the database.
- **Use Time/Use Volume:** These are available only from the Protocol screen, when defining a new protocol.

The following selections are available from the Manual screen:

- **Manual Setup:** This enables you to set the pump purge flow rate. Additionally, you can set an EGP split time period, select a QuadTec time constant, and set the SIM parameters. For the F10 pumps, you can specify a maximum purge rate of 10 ml/min; for the F40 pumps, you can specify a maximum purge rate of 40 ml/min.
- **Buffer Blender Setup:** This is available only when the Maximizer is used with the system. This allows you to switch between buffer blending and non-buffer blending modes. In buffer blending mode, a window listing buffer systems available for use is displayed. From this window a user can enter one or two point pH corrections if needed.

Table 5-8. Window Drop-down Menu

● BioLogic Duo-Flow - <user name> - <project name> - <method name>						
File	Edit	View	Utilities	Options	Window	Help
						1 BioLogic Online - <method name>
						2 BioLogic Offline - <method name>

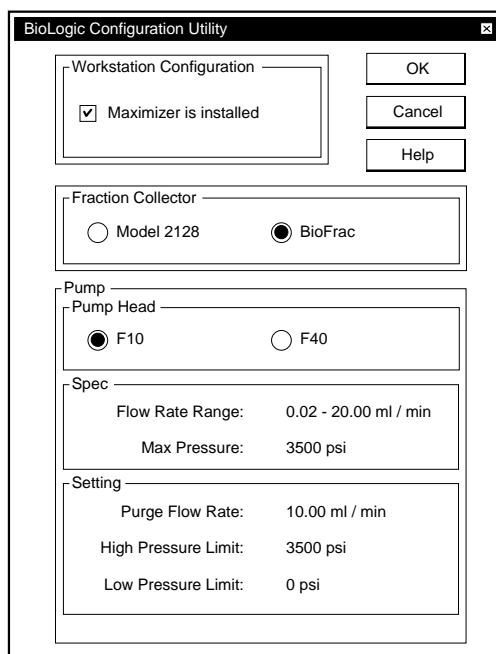
The BioLogic DuoFlow software allows you to continue working while a run is in progress (Offline). A complete discussion of this function is provided in section 7.4.2, Working Offline, page 7-27.

- **BioLogic Online:** Allows you to observe and control the run in progress.
 - **BioLogic Offline:** Allows you to write a protocol, analyze data, and export data while a separate run is in progress.
-

5.4 BIOLOGIC CONFIGURATION UTILITY SOFTWARE

The BioLogic Configuration utility is used anytime the pumpheads are changed or a Maximizer is installed. It is also used to choose between Bio-Rad's BioFrac and Model 2128 fraction collectors. To run this software:

1. Exit the BioLogic DuoFlow software by selecting Exit from the File drop-down menu.
2. Double-click on the BioLogic Configuration icon.
3. In the BioLogic Configuration Utility window, indicate the pump that will be used with the system, whether or not the Maximizer will be used with the system, and the default Bio-Rad fraction collector (either the BioFrac or the Model 2128).
4. Exit the BioLogic Configuration Utility and double-click on the BioLogic DuoFlow icon to start the software.

**Figure 5-2. BioLogic Configuration Utility Software Screen**

6.0 INTRODUCTION TO THE BROWSER SCREEN

The Browser is the organizational tool for Users, Projects, and chromatography data. It is a database that is displayed as a tree hierarchy which can be sorted by Users, Projects, Methods, and Runs. Figure 6-1 shows the layout of the Browser screen. A User must be selected or a new user added to the list and then selected in order to gain access to any program function except Manual. **All menus and tool bar functions will be grayed out and inaccessible until you select, or enter a User name in the Browser, refer to chapter 6, page 6-1.**

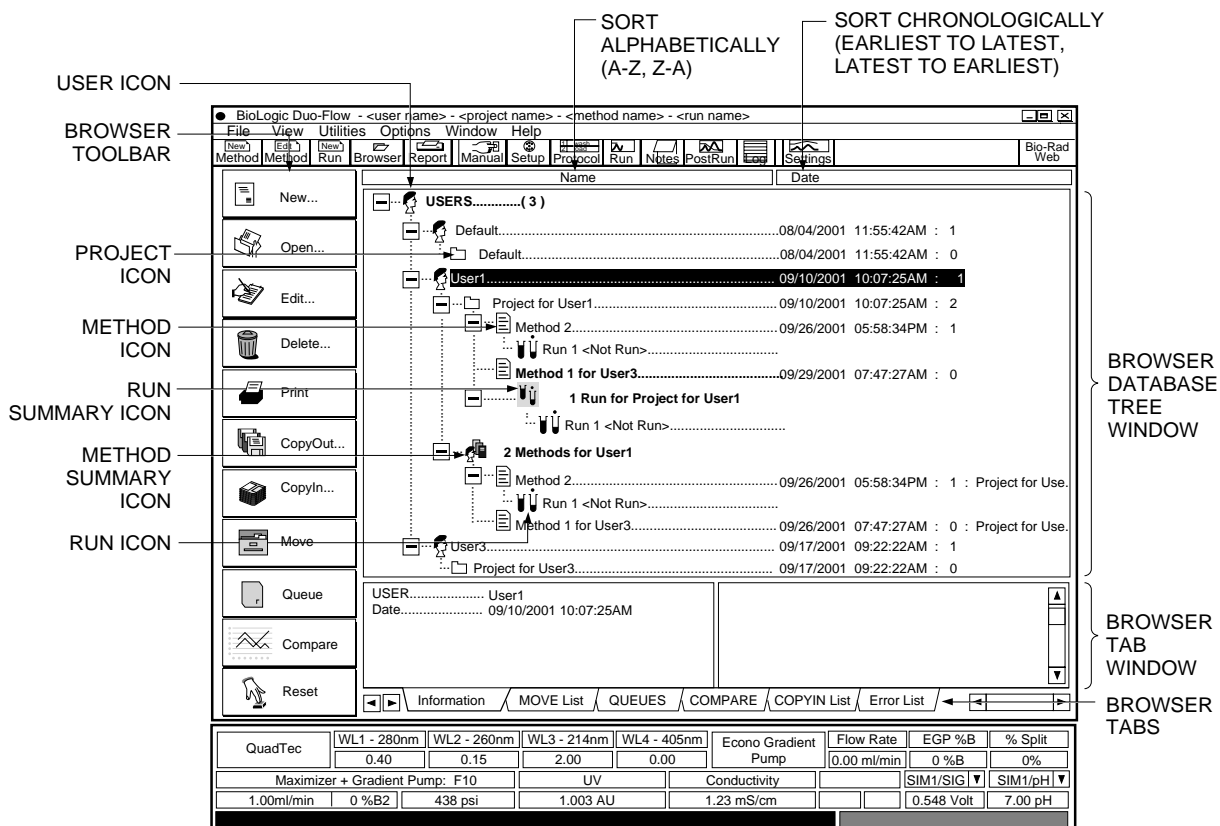


Figure 6-1. The Browser Screen

6.1 OVERVIEW

The Browser screen displays the following information and controls:

- **Collapsing/expanding the tree hierarchy:**



Click on this icon in the database tree to collapse the listing for a User, Project, Method, or run folder.



Click on this icon in the database tree to expand the listing for a User, Pproject, Method, or run folder.



Updates and refreshes the Browser screen by collapsing all folders to the top Users icon.

- **Icon colors in the database tree indicate the following:**

Green: Currently open in the active window. The active window may be the online window or the off-line window. For more detailed information about online and offline, refer to the discussion in Section 7.4.2, page 7-27, Working Offline.

Red: Currently open in the window that is not active. This condition is displayed during off-line use: it applies to the method and run data for the window that is not active (online or offline). Items with a red check mark indicate that item is in the Move List or is marked for deletion.

Blue (profile): User folder

Yellow: Project folder

Blue (text page): Method folder

Blue (test tubes): Runs

- **Bolded text in the database tree applies to the following:**

Top level folders: Typically this applies to the USERS top level folder, which lists all Users in the system. By selecting Browser Settings from the Options menu, you can use "Starting Browser Selections" to move projects, methods, and runs to the top level of the tree hierarchy where they will be bolded.

Summary information: The total number of methods are listed for each user, and the total number of runs are provided for each user project folder. You cannot delete summary information.

Browser screen controls and information

- **Browser toolbar:** Located down the left side of the screen, control the different Browser functions. They are discussed in greater detail on the following page.
- **Name and Date bars:** Toggle buttons located across the top of the database tree and allow you to sort the tree alphabetically or chronologically. For example, you can click on the Name button to sort from a to z, and then click again to sort from z to a. If you click on the Date button to sort from the latest listing, then clicking again sorts from the earliest listing.
- **Browser Tabs and Browser Tab window:** The Browser tabs at the bottom of the Browser screen control the information to be displayed in the Browser Tab window. The following tabs are available:

Information tab: Displays two panels in the Browser Tab window. The left panel shows details about the specific item selected; the right panel displays information about the next higher level item to which the item belongs.

MOVE List tab: Displays the list of all items selected using the Move button to be moved from one place to another in the database list. Items selected to be moved are indicated by a red checkmark in the database tree. Projects can be moved from one user to another; methods can be moved from one project to another. When a project is moved, it takes all methods and runs associated with it; when a method is moved, it takes all runs associated with it. The procedure for moving an item is on page 6-5.

Queue tab: Displays the methods in the Browser that have been placed in a Queue. The sequence of methods in a queue can be changed by dragging and dropping. Refer to section 6.2 page 6-8 for running a queued method.

Compare tab: Displays the runs selected or highlighted in the Browser for comparison. Refer to section 6.4, page 6-11 for running Trace Compare.

COPYIN List tab: Displays the list of all items selected to be copied in (restored) to the database from an archived data file or disk. The procedure for copying in items is discussed on page 6-4.

Error List tab: Displays discrepancies that exist between methods in a Queue. These discrepancies must be corrected to run the queue.

Browser toolbar buttons

New...	To create a new User, Project, Method, Run, Queue or Compare depending on which icon within the Browser is highlighted.
Open...	Allows you to open a selected Method or Run from the database tree. Opening a Run allows you to view, analyze, and print the run data.
Edit...	<p>Select a User or Project and Edit allows you to change that User name or Project name and/or description.</p> <p>Select a Method or Run to be edited. The edited Method will be numbered sequentially and placed in the Browser as a new method.</p> <p>Summary items are not editable.</p>
Delete	<p>Eliminates Users, Projects, Methods and Runs from the Browser. To delete a Method, you must first delete all of the Runs associated with the method.</p> <p>You cannot delete methods or runs from method and run summaries.</p>
Print	To generate and print the report for a run.
CopyOut...	<p>Allows you to copy Methods and Runs to floppy disk or to another location on your computer's hard drive. The CopyOut function is used for backup and archiving purposes and to transfer data between DuoFlow systems.</p> <p>Copied out files are stored in a file with the default name biologic.zip, which is a compressed file format to conserve disk space. The copy out function copies the User, Project, and Method names associated with each.</p> <p>Initiating a CopyOut to floppy disk completely erases the contents of the floppy disk.</p> <p>To copy out, highlight the runs you wish to copy out. Press the CopyOut... button on the Browser toolbar. A dialog box appears that allows you to select the destination of the .ZIB file (either the hard drive or a floppy disk). Choose the destination and press OK.</p>

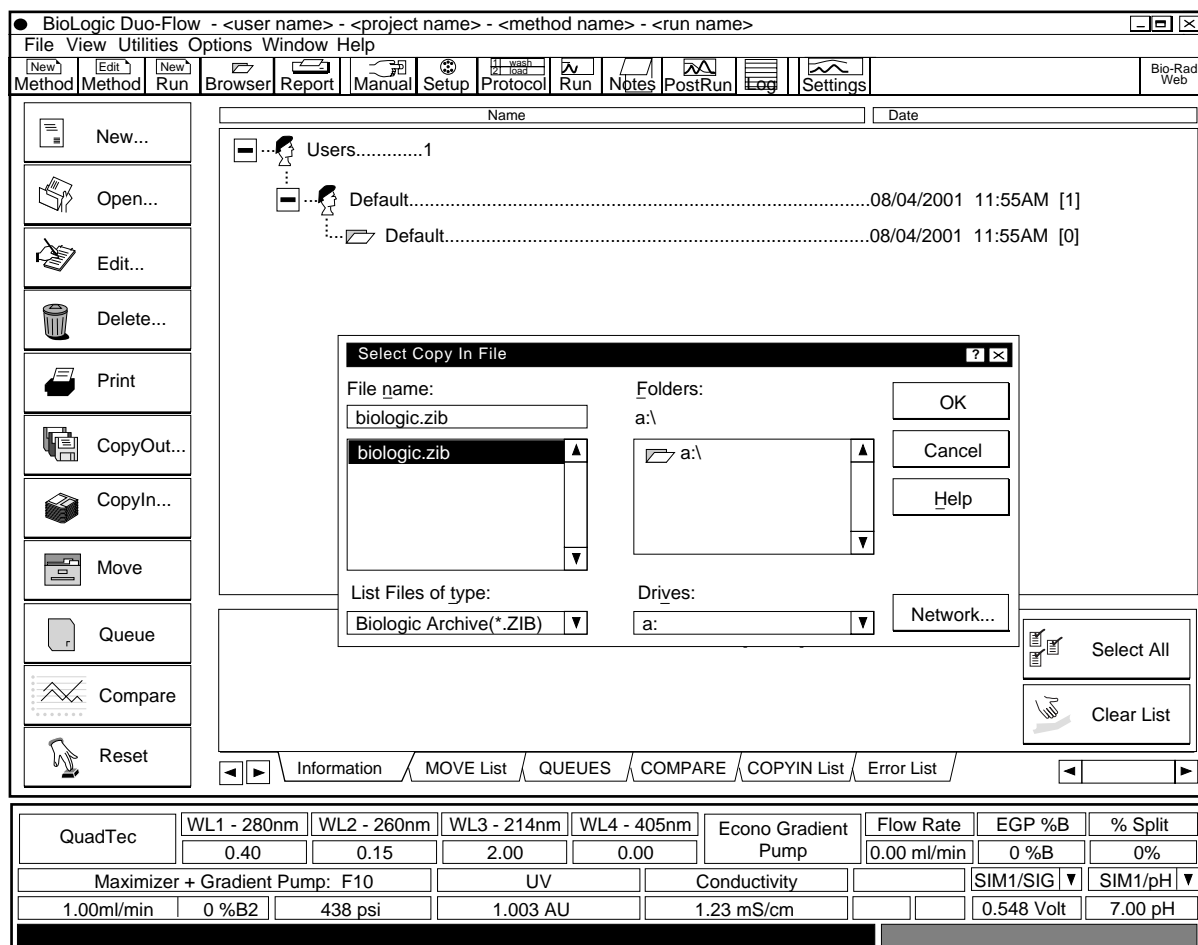


Figure 6-2. The CopyIn Window

CopyIn...

Allows you to copy in Methods and Runs from a backed-up or archived .ZIB file. Refer to CopyOut page 6-3. To copy in:

- Press the CopyIn... button and select the .ZIB file you wish to copy in. (Figure 6-2 above.)
- From the COPYIN List in the Browser Tab window, select the desired Methods and Runs.

All Methods and Runs in the file are displayed in the COPYIN List. (Figure 6-3, page 6-5.)

From the database tree window, click the left mouse button to select the Project to which you want the Methods and Runs to be copied.

- Again, click on the CopyIn... button (or click on the right mouse button) and select Copy to <project name>. Specify where to copy the file to.

Clicking the right mouse button displays all the available Browser toolbar buttons for the highlighted selection.

- Once you finish the copy in procedure, use the Clear List button on the Browser tab window to delete the remaining methods and runs in the COPYIN List.

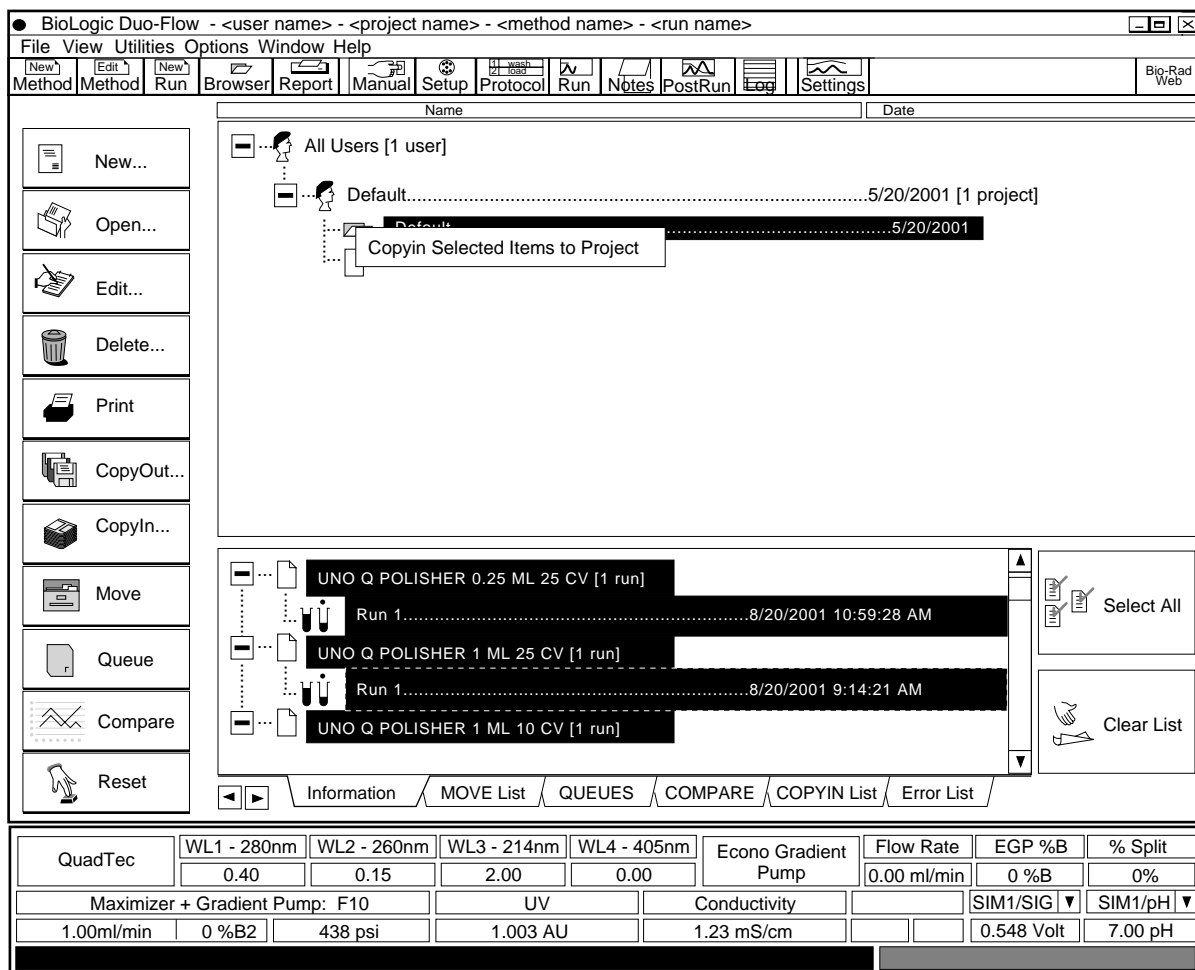


Figure 6-3. CopyIn with Information in Browser Tab Window

Move

To transfer a selected Project or Method from one location to another. For example, a Project can be moved from one User to another, and a Method (and its runs) can be moved from one project to another. Individual runs cannot be moved. To move an item:

- Highlight the item in the database tree you want to move. You may highlight a Project or a Method.
- Click once on the Move button on the Browser toolbar. The items to be moved will appear in the MOVE List in the Browser tab window.
- From the MOVE List, select the items you want to move.
- From the database tree, select the new destination for the items you are moving.
- Again click on the Move button. The items will automatically move to the destination. Hint: Alternatively, highlight the destination icon, right-mouse click and select the "Move Selected" message with a left-mouse click.
- Once you finish the move procedure, use the Clear List button on the Browser toolbar to delete the remaining methods and runs in the MOVE List.

Queue

Places methods in a queue. Highlight the method(s) you wish to place in a queue and select Queue. The methods will appear in the tab screen. Refer to section 6.2, page 6-8 for detailed description of queuing.

- Compare** Places runs in a list for overlay comparison. Highlight the Run(s) you wish to compare and select Compare. The run will appear in the tab screen. See Section 6.3 for detailed description of trace compare.
- Reset** Updates and refreshes the Browser screen by collapsing all folders to the single user icon.

There are several options available for how information is displayed in the Browser screen. Select Browser Settings from the Options menu, discussed in Table 5-7, page 5-11, Options Drop-down Menu. The Set Browser Options window provides the following options:

- **Enable Projects:** A summary of all Projects, regardless of User, will be listed in the Browser.
- **Enable Methods:** A summary of all Methods, regardless of User or Projects, will be listed in the Browser.
- **Enable Runs:** A summary of all Runs will be listed in the Browser.
- **Enable User Methods:** A summary of all Methods for the specified User will be listed in the Browser.
- **Enable Project Runs:** A summary of all Project Runs will be listed in the Browser.

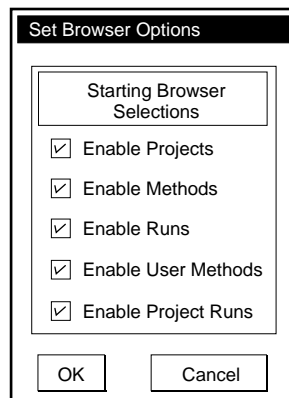


Figure 6-4. Set Browser Options Window

Procedures for creating a run in the Browser

1. Enter a New User.

Enter a new User to allow you to define your Methods and to group Projects, Methods, and Runs within the Browser.

- a. Click once on the Users icon in the Browser.
- b. Click once on the New... button and select New User. You will be prompted for a new User Name. Enter the name you would like to use.
- c. Press OK to accept the new name.

If you define a new User, you must also assign a Project to the User before writing a new Method.

Hint: Alternatively, highlight a User icon, right-mouse click, and select New User with a left-mouse click.

2. Define a New Project.

The Browser is further segmented into Projects within a User's domain. To define a new project:

- a. With the new User Name highlighted, click once on the New... button.
- b. Select New Project. Enter the project name and description.
- c. Press OK to accept the new name.

Hint: Alternatively, highlight the User icon, right-mouse click, and select New Project with a left-mouse click.

3. Write a New Method.

To begin writing a new method from the Browser:

- a. Select the Project folder or a Method in the Project folder where you want the new method to be located.
- b. Click once on the New... button.
- c. Select New Method. Enter the method name and press OK. This transfers you to the hardware Setup screen.

Hints: 1. Alternatively, highlight the Project icon, right-mouse click, and select New Method with a left-mouse click.

2. You can also use the New Method button in the toolbar. By default the system assumes that the current user and project shown in the window title bar is the path for the new method.

4. Name a New Run in a previously defined Method.

To start a new run from the Browser:

- a. Select a Method or a Run in the Method where you want the new Run to be located.
- b. Click once on the New... button.
- c. Select New Run. Enter the Run name and press OK. The Run screen appears. Pressing the Start button in the toolbar will begin your sample run.

Hints: 1. Alternatively, highlight the Method icon, right-mouse click, and select New Run with a left-mouse click.

2. You can also use the New Run button in the toolbar. By default the system assumes that the current User, Project, and method shown in the window title bar is the path for the new Run.

6.2 CREATING AND RUNNING A QUEUE

Queue allows you to run multiple methods in sequence. **Methods placed in a queue must have identical hardware setups or the system will not permit them to run.**

The screenshot shows the BioLogic Duo-Flow Browser window. The top menu bar includes File, Edit, View, Utilities, Options, Window, and Help. The toolbar contains icons for New, Edit, Run, Browser, Report, Manual, Setup, Protocol, Run, Notes, PostRun, Log, and Settings. The left sidebar has buttons for New..., Open..., Edit..., Delete..., Print, CopyOut..., CopyIn..., Move, Queue, Compare, and Reset. The main area displays a tree view of the system hierarchy under 'Name' and 'Date' columns. The 'Queue' tab is selected, showing a list of queues. The 'Demo Queue with QuadTec.1' is selected, and its details are shown in the bottom panel.

QUEUE NAME

Queue Name	Date	Count
ChemLab.....	03/02/2001 10:04:56AM	1
Project for ChemLab.....	01/07/2001 07:08:34PM	0
Mary Jones.....	01/13/2001 07:34:43AM	1
Project for Mary Jones.....	03/13/2001 07:35:01AM	0
Joe Smith.....	03/27/2001 05:05:48PM	1
Project for Joe Smith.....	03/28/2001 02:49:39PM	0
Default.....	03/27/2001 05:05:48PM	1
Default.....	03/28/2001 02:49:39PM	0
Demo Chromatography.....	02/13/2001 07:34:43AM	1
Project for Demo Chromatography.....	03/13/2001 07:35:01AM	3
Demonstration Queue.....	03/27/2001 05:05:48PM	1
Demonstration Queue [1].....	03/27/2001 05:05:48PM	4
Run: [Q] Queue Method #3.....	03/28/2001 02:49:39PM	
Run: [Q] Queue Method #2.....	03/28/2001 02:42:54PM	
Run: [Q] Queue Method #1.....	03/28/2001 02:36:06PM	
standard UV detector.....	03/27/2001 05:05:48PM	1
Sample Run.....	03/28/2001 02:49:39PM	
QuadTec with Starter Kit (#750-0135).....	03/27/2001 05:05:48PM	1
Sample Run.....	03/28/2001 02:49:39PM	

Methods in Queue:

Queue Name	Date	Count
1 [Q] QuadTec Starter Kit.2.....	03/27/2001 05:05:59PM	
2 [Q] QuadTec Starter Kit.1.....	03/27/2001 05:05:59PM	
3 [Q] QuadTec Starter Kit.....	03/27/2001 05:05:59PM	

Hardware Parameters:

Parameter	Value
QuadTec	WL1 - 280nm, WL2 - 260nm, WL3 - 214nm, WL4 - 405nm, Econo Gradient Pump, Flow Rate: 0.00 ml/min, EGP %B: 0 %B, % Split: 0%
Maximizer + Gradient Pump: F10	UV, Conductivity, SIM1/SIG, SIM1/pH
1.00ml/min, 0 %B2, 438 psi	1.003 AU, 1.23 mS/cm, 0.548 Volt, 7.00 pH

METHODS IN QUEUE

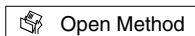
Figure 6-5. Queues Displayed in the Browser Window

To create a queue,

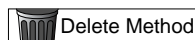
1. In the Browser screen select a User or enter a new User name.
2. Select a Project or enter a new Project name.
3. Select the NEW icon from the left screen sidebar, and from the displayed menu select New Queue. Enter a name and description for your Queue and click OK. Your queue name will be listed under your User and Project name, and the Queue tab will appear at the bottom of the screen. (See Figure 6-5 above.)

4. To place methods in a Queue, highlight the method in the Browser and click the Queue icon on the sidebar. Repeat until all desired methods are in the project Queue. This places each method into your Project Queue and the Queue Tab window at the bottom of the screen.
 - Whenever you highlight the Queue icon under the Project Queue, the list of methods will appear in the Tab window.
 - Multiple methods may be selected in the Browser by either shift/click or control/click.
 - Methods will run in the sequence they are listed in the Tab window. The run order of the methods can be changed by dragging and dropping methods in the queue in the Tab window.
5. After all methods are listed in the project queue, click the Verify Queue button in the right sidebar of the Tab window to confirm that all methods meet the necessary criteria to be run.
 - If the methods meet the necessary run criteria to run correctly, "Methods were successfully verified" will appear.
 - If methods do not meet the necessary run criteria, a list of errors will appear. Errors will appear if methods are not compatible to run in sequence or the hardware Setup screens are not identical.
 - To run your queue, click the Run Queue icon in the right sidebar of the Tab window.

Description of the Queue Icons



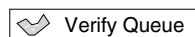
Open Method: Opens a highlighted method in the Protocol screen.



Delete Method: Deletes a highlighted method from the queue. This is displayed only prior to the queue running.



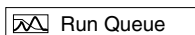
Edit Method: Opens a highlighted method in the Protocol screen to be edited. This is displayed only when the Queue has completed its run. Queued methods cannot be edited after the run has started.



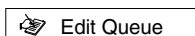
Verify Queue: Checks methods in a queue to assure there is not a conflict in the method Protocol and Setups to prevent running.

If there is a conflict, a list of errors will appear.

If methods are compatible, the message "Methods were successfully verified," appears.



Run Queue: Starts a run queue.



Edit Queue: Permits the editing of a Queue.

6.3 CREATING AND VIEWING A COMPARE

Trace Compare allows you to view and compare an unlimited number of chromatogram runs simultaneously in either tile or overlay mode.

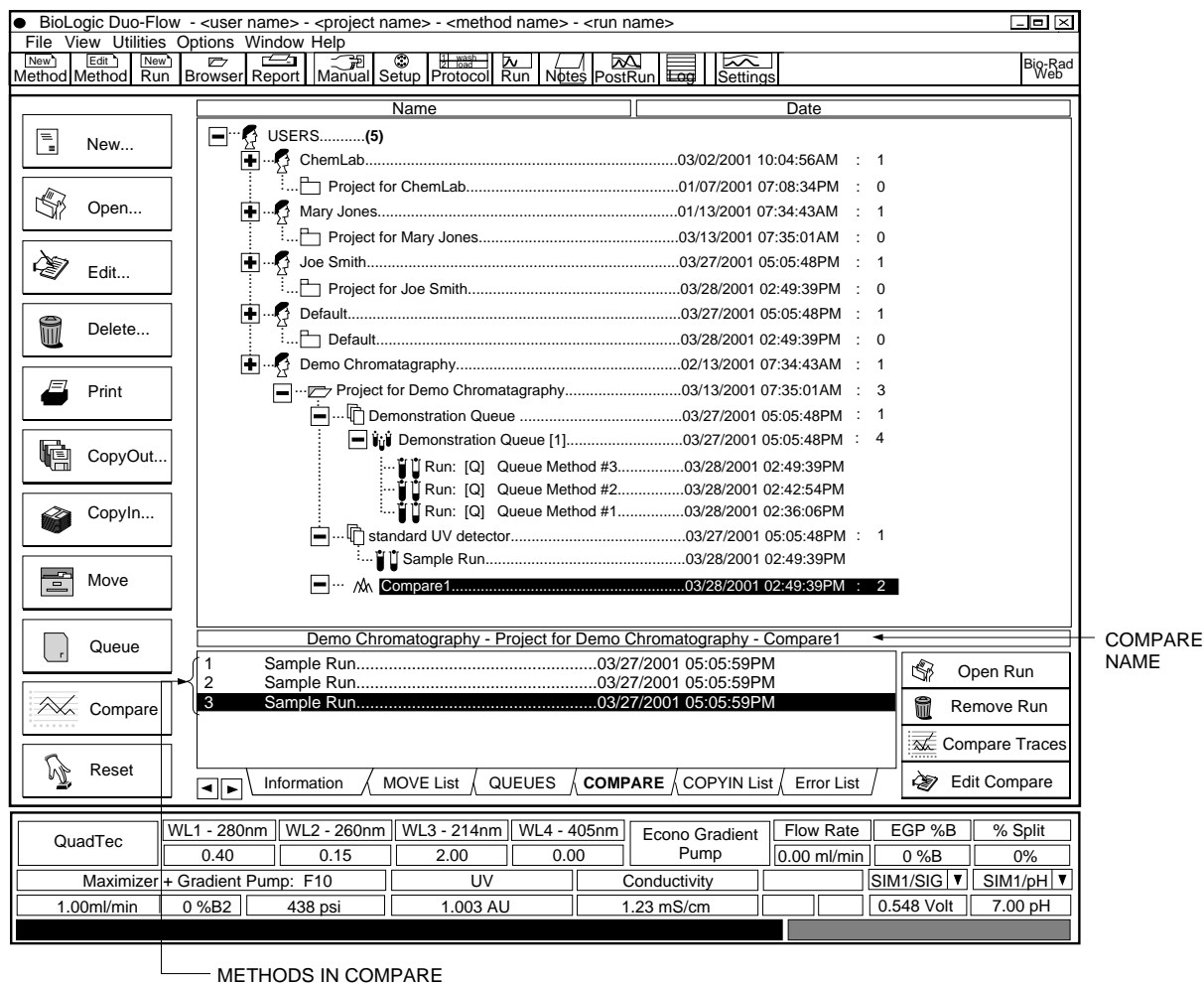
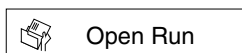


Figure 6-6. Compare Displayed in the Browser Window

To create a compare,

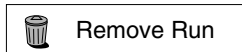
1. In the Browser screen select the user name and open the method that contains the runs you wish to compare.
2. Select the **NEW** icon from the left screen sidebar, and from the displayed menu select **New Compare**.
3. To place runs into the compare dialog, highlight the runs and click the **Compare** icon on the sidebar. Repeat until all desired runs are in the lower screen Project Compare window. This places each run into your Project Compare and the Compare Tab window at the bottom of the screen.
 - When you highlight the **Compare** icon under the Project Compare, the list of runs will appear in the Tab window.
 - Multiple methods may be selected in the Browser by either shift/click or control/click.

Description of the Compare Icons



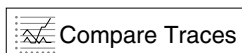
Open Run

Open Run: Opens a highlighted run in the PostRun screen.



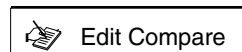
Remove Run

Remove Run: Deletes a highlighted method from the compare.



Compare Traces

Compare Traces: Opens the Trace Compare window.



Edit Compare

Edit Compare: Permits editing of the compare name, description and author.

6.4 TRACE COMPARE

Trace Compare is a tool for simultaneously visualizing and comparing chromatography data. Figure 6-7 shows the layout of the TraceCompare window.

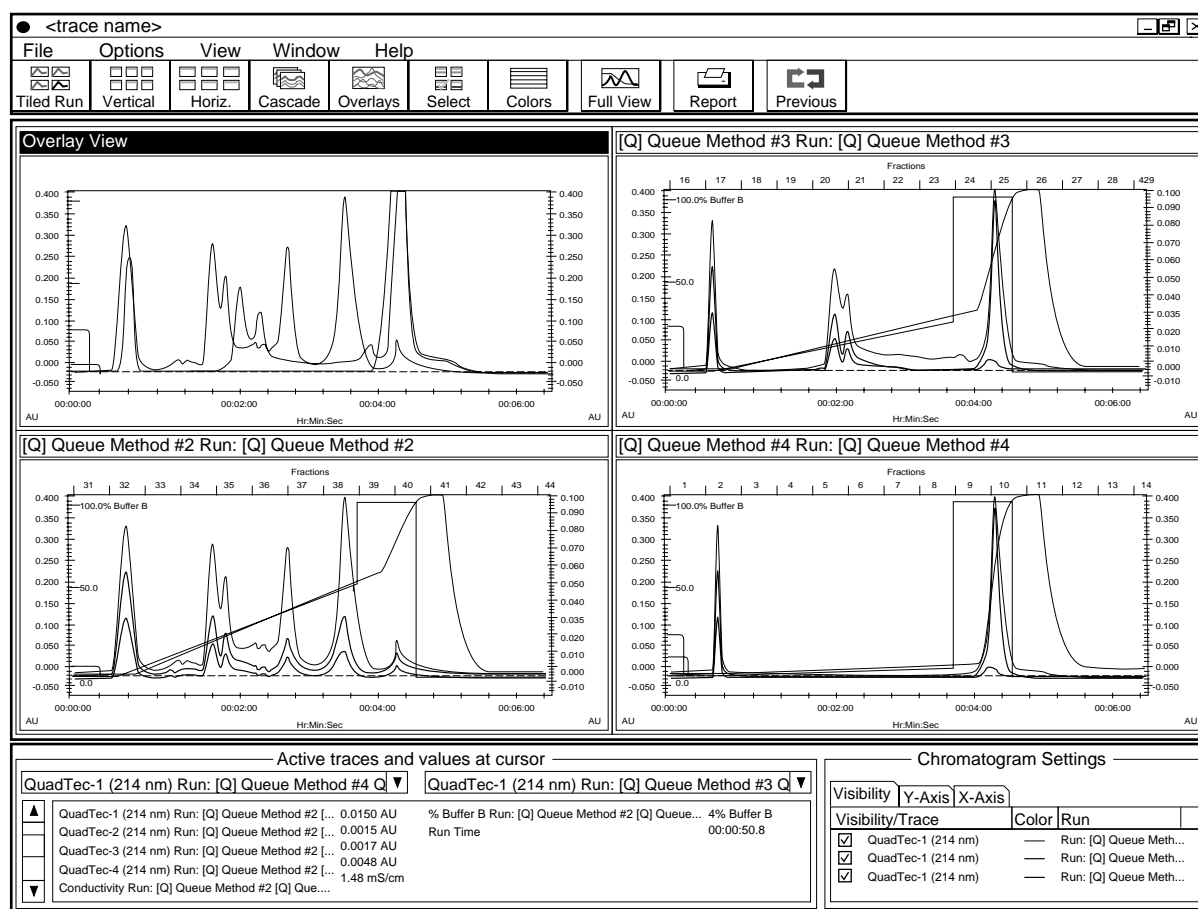

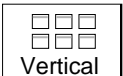










Figure 6-7. Layout of the Trace Compare Window

6.4.1 Toolbar Buttons

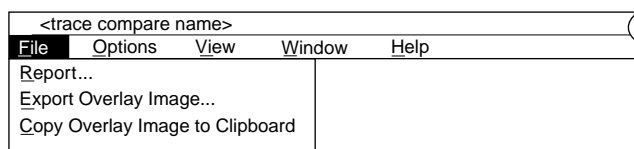
The function of each toolbar button is provided in Table 6-1.

Table 6-1.
Toolbar Buttons

Button	Description
 Tiled Run	Tiled Run: Places chromatogram in Trace Compare in tile view.
 Vertical	Vertical: Displays chromatogram windows vertically. Active only in tile view.
 Horiz.	Horiz.: Displays chromatogram windows horizontally. Active only in tile view.
 Cascade	Cascade: Displays chromatograms in Trace Compare in cascade view.
 Overlays	Overlays: Places chromatogram in Trace Compare in overlay view.
 Select	Select: Allows you to select which traces will appear in overlay view.
 Colors	Color: Toggles between two trace coloring schemes: Color by device type or color each trace uniquely.
 Full View	Full View: Expands the currently active chromatogram to full scale.
 Report	Report: Allows you to preview and print a report of the currently open compare, including an Overlay Report, Trace Compare Report: Chromatograms, Trace Compare Report:Legends and Trace Compare Report: Summary.
 Previous	Previous: Closes Trace Compare and returns to the browser.

6.4.2 Drop-down Menus

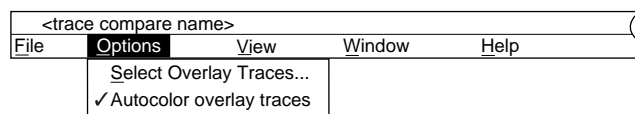
Table 6-2.
File Drop-down Menu



The File menu consists of the following:

- **Report:** Allows you to preview and print a report of the currently open compare, including an Overlay Report, Trace Compare Report: Chromatograms, Trace Compare Report:Legends and Trace Compare Report:Summary.
 - **Export Overlay Image:** Allows you to export the overlay view to a windows metafile (.wmf) or enhanced metafile (.emf).
 - **Copy Overlay Image to Clipboard:** Allows you to copy the overlay view to the clipboard for pasting into other applications.
-

Table 6-3.
Options Drop-down Menu



The Options menu consists of the following:

- **Options/Select Overlay Traces...:** Allows you to select which traces will appear in overlay view.
 - **AutoColor Overlay Traces...:** Toggles between coloring each trace a different color or coloring each trace by trace device type.
-

Table 6-4.
View Drop-down Menu

<trace compare name>				
File	Options	View	Window	Help
			Switch to BioLogic	
			✓ Tiled Run Chromatograms	
			Overlay Chromatogram	
			All Chromatograms	
			Legend...	
			Full View	
			Full View for All Chromatograms	

The View menu consists of the following:

- **Switch to BioLogic:** Closes Trace Compare and returns to the browser.
- **Overlay Chromatogram:** Places Trace Compare in overlay view.
- **All Chromatograms:** Places Trace Compare in tile view and displays all chromatograms including the overlay view.
- **Legend:** Displays the legend for the the current view.
- **Full View:** Expands the currently active chromatogram.
- **Full View for All Chromatograms:** Expands all chromatograms.

Table 6-5.
Window Drop-down Menu

<trace compare name>				
File	Options	View	Window	Help
			Tile Views Vertically	
			Tile Views Horizontally	
			Cascade Views	

The Window menu consists of the following:

- **Tile Views Vertical:** Displays chromatograms vertically in tile view.
- **Tile Views Horizontal:** Displays chromatograms windows horizontally in tile view.
- **Cascade Views:** Displays chromatograms windows in cascade view.

6.4.3 Windows

Active Traces and Values at Cursor

The Active traces and values in the "at cursor" window located in the upper left of the post run screen, contain two drop-down menus that control which trace axis is displayed on the left and right sides of the active chromatogram. The scroll bars on the left and right sides of the information box control the scaling of the trace associated with the left or right drop-down menu. When the vertical crosshatch bar is dragged across a chromatogram, the values relating to the baseline or peak underneath the crosshatch is reflected in the upper left corner of the screen. The value for each trace at the cursor position is shown in the dialog.

Chromatogram Settings Tab

The Chromatogram Settings window contains the following tabs to manipulate the display of the chromatogram:

Visibility	<p>In Tile mode each trace device type is displayed along with a check box, legend, and a run name. Checking or unchecking the boxes causes the traces to be displayed or hidden.</p> <p>In Overlay view only the traces selected in "Options/Select Overlay Traces..." or by the Select button are shown in the chromatogram settings dialog.</p> <p>The currently active trace is highlighted in blue.</p>
Y-Axis	<p>Allows you to set the Baseline and Axis Max values for the currently active trace. The active trace is selected in the Visibility tab.</p>
X-Axis	<p>Allows you to set the Start Time and End Time for the currently active chromatogram.</p>

7.0 MODES OF OPERATION

There are two primary modes of system operation: the user can operate the system manually (from the Manual screen) or through the use of a user-defined method. Each of these modes of operation is discussed below, as well as the screens involved in setting up, defining, and running a method.

- Operating the system using the Manual screen: This allows you to individually control the devices and instruments connected to the Workstation. Examples of its use include purging the pumps and equilibrating the columns. For further discussion, refer to Section 7.1.
- Operating the system using a user-defined method: This involves the following:

Creating a new method or selecting an existing method.

Setup screen: Allows you to define the instruments and devices to be used for any method.

Protocol screen: To define each of the steps to be run during a method. Most steps are programmed using a dialog box to enter the parameter values. Note that the number of the step being programmed is displayed in the upper left corner of the dialog box.

Run screen: To run a method or view a run in progress.

Start: Starts a selected run.

Note: You may move through the four screens by activating buttons on the system toolbar as long as a run is not in progress. Those functions available during a run are discussed in section 7.4, Run Screen.

The relationship between the two modes of operation, as well as the screens involved in defining and running a method, is illustrated in Figure 7-1 below.

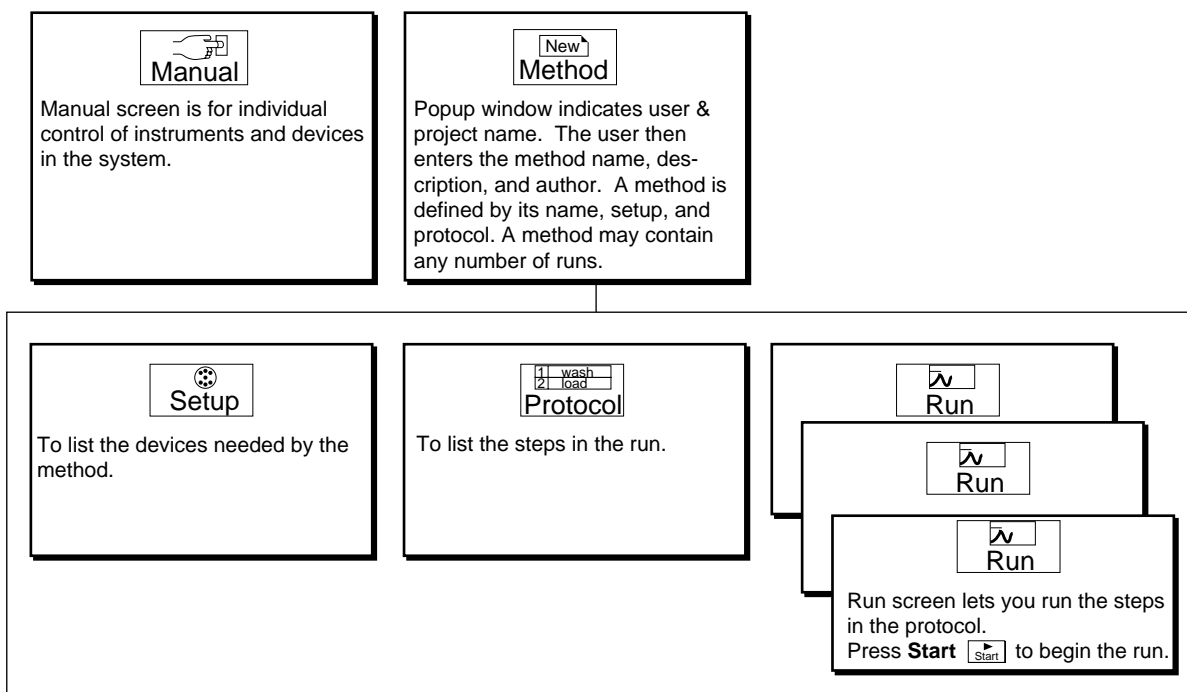
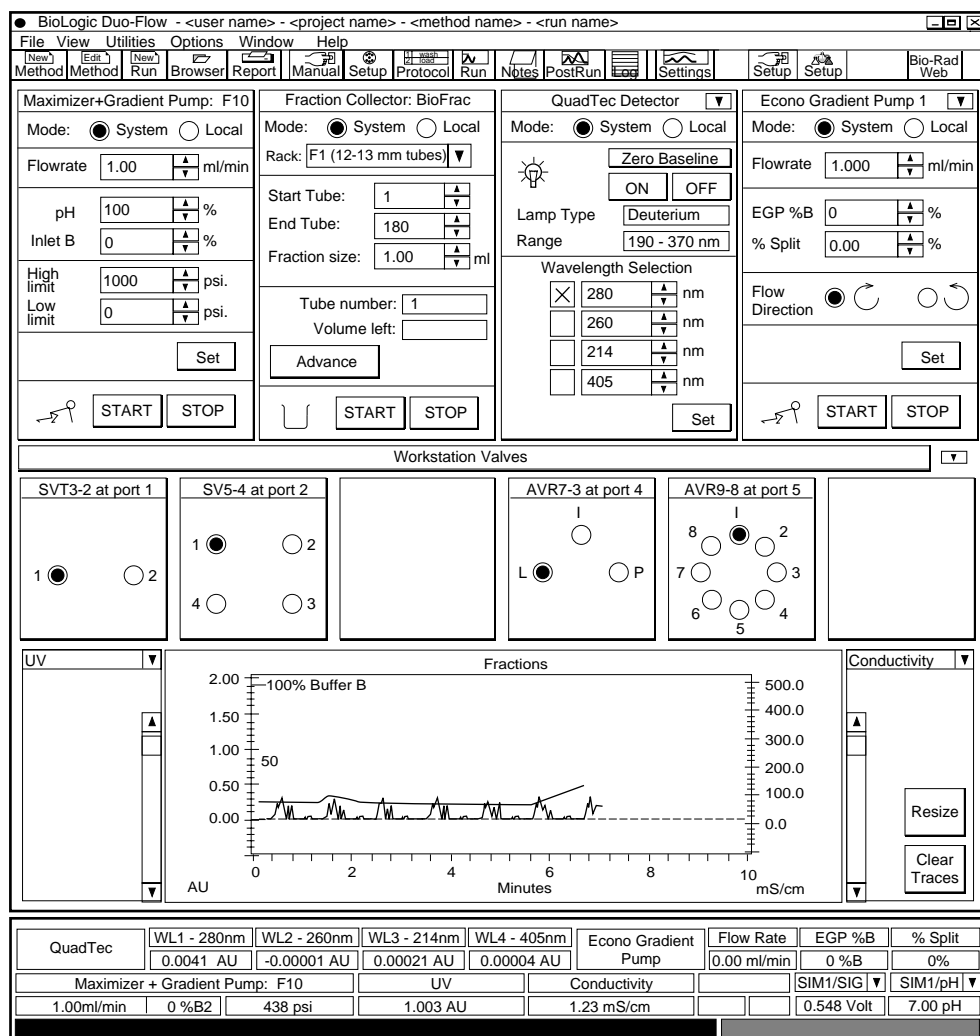


Figure 7-1. Relationships between Modes of Operation

7.1 MANUAL SCREEN

The Manual screen displays all peripheral hardware connected to the Workstation. Each device may be manually manipulated from this screen. Detector activity and pump pressure is displayed in a realtime chromatogram for baseline tracking.

Note: If an instrument is connected via the instrument bus but does not appear in the Manual screen, check that its power switch is on and that the instrument bus cable connection is secure.



CONTENTS
MAY VARY
DEPENDENT ON
CONFIGURATION

Figure 7-2. Manual Screen, showing a Maximizer, BioFrac Fraction Collector, QuadTec Detector, Econo Gradient Pump, and Four Valves

The Maximizer, BioFrac and Model 2128 fraction collectors, QuadTec detector, and Econo Gradient Pump have two modes of operation: **System**, in which the full functionality of the unit is available from the DuoFlow system software, and **Local**, in which control of the instrument is from its front panel faceplate.

From the Manual screen you can control the following:

- **Workstation Pumps:** This control panel allows you to control the composition of the mobile phase being pumped, set the flow rate and pressure limits, and start/stop the pump. (User-defined pressure limit settings is discussed in section 7.1.1.). If the pressure limit is exceeded, the pumps stop.

Changing pressure limits requires pressing either the **Start** or **Set** buttons. When the pump is running, changing any parameters requires pressing the **Set** button.

- **Fraction Collector:** When a Bio-Rad BioFrac or Model 2128 fraction collector is connected, the control panel displays the "Tube number," which is the tube currently under the drop-head (assuming the run started at tube 1), and the "Volume left," which is the volume remaining to be delivered. There are also toggle buttons for **Start** and **Stop** and a button for **Advance**. The Tube number is a display field only.

Fraction collection is always by **Volume**. Collection by Drop or Time is not supported by the DuoFlow software.

- **Econo Gradient Pump:** For this optional peristaltic pump the control panel allows you to select a flow rate, determine percentage of flow divided by the EGP splitter valve, select a flow direction and start and stop the pump. Specific details for operation will be in the EGP instruction manual.
- **QuadTec and UV Detectors:** The down arrow in the upper right corner of the control panel toggles between the QuadTec and the UV detector control panels.

QuadTec detector: The control panel allows you to select and zero 4 wavelengths and to turn the lamp on and off. Specific details for operation of the QuadTec detector can be found in its instruction manual.

UV detector: The control panel provides access to the UV detector, chart recorder and indicates Signal Import Module status. It allows you to turn the UV lamp on and off and to zero the baseline.

- **Chart Recorder:** If you are using the UV detector and Conductivity monitor, you can set the chart recorder full scale ranges, start/stop the recorder, and send an event mark. The full scale ranges set here apply to the chart recorder and are not reflected in chromatograms displayed on the computer screen.
- **Signal Import Module (SIM 1 and 2):** This panel indicates the presence of up to two Signal Import Modules (SIM). The signal from an analog instrument connected to the SIM is digitized so that the instrument can be monitored from the DuoFlow system. Instruments that may be connected include any detection source, such as a variable wavelength UV detector, a pH meter, a refractive index detector, or a fluorescence detector. For more information, refer to Chapter 2.9.6 and to the separate instruction sheet for the SIM module.
- **Valves:** Allows you to control the valve position of any of the DuoFlow solenoid or automated valves connected to the Workstation or Maximizer. To access valves connected to the Maximizer, select the **down arrow** toggle switch in the upper right corner of the valves panel.
- **Resize** enlarges the chromatogram screen.
- **Clear traces** renews the chromatogram screen.

The Manual screen chromatogram displays the system output for up to 8 instrument traces from any of the following: UV detector, conductivity, pH, pressure, theoretical %B concentration, four QuadTec wavelengths, and detector traces acquired via the System Interface Module (SIM). Use the scroll bars, the **Settings** button in the toolbar, or the **Chromatogram Settings** selection under the **Options** drop-down menu to change the x- and y-axes settings. The drop-down menus on either side of the chromatogram permit selection of a trace for adjustment to its sensitivity reading. The **Resize** button enlarges the Manual screen chromatogram display.

7.1.1 Pressure Limit Settings

The DuoFlow system is designed for pressures to 3500 psi, with the F10 pumpheads installed. The F40 pumpheads are rated to 1000 psi at 40 ml/min. If you will be using a column designed for lower pressures, you can set a lower pressure limit for the system. From the Gradient Pump panel, set the high limit to the appropriate value for the column. Set a low limit that is 6 psi or greater (the system does not allow minimum pressure below 6 psi) to protect the system from pumping air if it runs out of buffers or mobile phase. If the pressure limits are exceeded, the pumps stop.

Note: Changing pressure limits requires pressing either the **Start** or **Set** buttons.

7.2 SETUP SCREEN

The Setup screen is used to select instruments/devices to be used in the protocol for a particular user-defined method. Each method has its own setup defined for it. If you routinely use the same setup for different methods, you can select **Save setup...** from the File drop-down menu to save a setup for use with another method or to define it as the default. This eliminates the need to build a new setup for a method.

In Figure 7-3, Setup screen devices are chosen from the left hand “Available Devices” box. A dialog box appears, allowing you to label the device in the method. For example, an AVR7-3 may be selected and then defined as a Sample Inject Valve. Use the **Delete** button in the Toolbar to remove a device from the Devices in the Setup window. In order to **run** your method, these devices must be connected to the rear of the Workstation and they must be powered on.

Note: Devices may not be properly connected to the system but may be included in the setup. The run will not start, however, until the devices are connected. An error message will appear on the Run screen.

QuadTec	WL1 - 280nm	WL2 - 260nm	WL3 - 214nm	WL4 - 405nm	Econo Gradient Pump	Flow Rate	EGP %B	% Split
	0.40	0.15	2.00	0.00		0.00 ml/min	0 %B	0%
Maximizer + Gradient Pump: F10					UV	Conductivity		SIM1/SIG ▼ SIM1/pH ▼
1.00ml/min	0 %B2	438 psi	1.003 AU	1.23 mS/cm			0.548 Volt	7.00 pH

Figure 7-3. Setup Screen

The following devices are listed in the Setup screen. Because the UV detector, Conductivity monitor, and AVR7-3 valve are standard with the DuoFlow system, they are always listed in the Devices in the Setup panel. To eliminate any of these, highlight it and click the **Delete** button.

- **Auxiliary Pump:** Allows you to use an auxiliary pump, such as the Model EP-1 Econo pump or the Econo Gradient Pump (EGP). See Chapters 2 and 8, for ways to use an auxiliary load pump.

- **Fraction Collector:** Selects a fraction collector. The selection options are a BioFrac, Model 2110, Model 2128, or generic (non-Bio-Rad) fraction collector. If you use threshold detection to initiate fraction collection, select either the detector in use or the Conductivity monitor. To enable the use of collection windows or threshold collection for a Model 2110 or generic fraction collector, it is necessary to assign an SVT3-2 valve as the fraction collector diverter valve.

Selecting the BioFrac or Model 2128 allows you to specify the rack type and number of racks. If the multiple rack option is selected, the system will pause at the appropriate time during the run and a yellow prompt will tell you to change the rack before continuing the run.

The BioFrac and Model 2128 also allow you to specify a delay volume. The Delay Volume function precisely synchronizes fraction collection with the UV signal on the chart recorder. It is defined as the tubing volume between the UV detector and the fraction collector drop-head. If you are using the recommended 1/16" (1.6 mm) OD, 0.020" (0.51 mm) ID Tefzel tubing, 1 cm of tubing has a volume of 2 μ l. The volume of the supplied backpressure regulator is 146 μ l

- **Buffer Blender:** There are a number of buffer systems for use with DuoFlow Maximizer systems. From the Buffer System window, select the desired buffer system. As a buffer system is selected, the pH range at 25° C, molarity at 100% B, temperature compensation coefficient, and recipe are displayed in lower boxes. Indicate if a pH correction is to be used, either single or two point. Buffer recipes may be printed by selecting **Print Report** from the File menu or the **Report** toolbar button. The selection includes a method option.
- **Detectors:** The UV detector and Conductivity monitor are provided with DuoFlow systems. The QuadTec detector from Bio-Rad is discussed in greater detail in its instruction manual. SIM/pH is for use with Bio-Rad's pH monitor and SIM (Signal Import Module). SIM Signal is for use with non-Bio-Rad detectors, such as refractive index and fluorescence detectors.

The **SIM Signal** button requires that you set the **Units** and the minimum and maximum for the **Units Range** and **Device Output Range (volts)**. Select the device number on the SIM to match the number displayed. Refer to the analog device's documentation for information on output voltages.
- **Valves (SVT3-2, SV5-4, AVR7-3, and AVR9-8):** The DuoFlow system's automatic valves can be configured in a number of different ways depending on their function in a method. Table 7-1 discusses how each of the valves can be set up.
- **Gradient Pump:** Inlets A and B may be renamed by overwriting "Buffer A" and "Buffer B" with the description of the buffer. When the Maximizer is used, the inlets become A1, A2, B1, and B2.

Valve Function and Naming

One of the most powerful features of the DuoFlow Software is the ability to define the specific purpose of the automated valves and to name each valve position. This greatly simplifies programming individual steps in the Protocol screen.

For example:

- The most common use of an AVR7-3 valve is as a sample inject valve. Once an AVR7-3 valve has been assigned to this function, the software controls all of its valve changes based on the programmed sample injection step.
- The most common use of a SVT3-2 valve will be as a fraction collector diverter valve with a Model 2110 or generic collector. Once an SVT3-2 valve has been assigned to this function, the software controls eluant diversion based on the programmed fraction collection scheme.

In summary, you can identify the following as applying to all valves:

- **Valve Name/Function.** What is the valve to be used for? Select from the drop-down list box or input a unique function that you will recognize when the particular valve is selected in the Protocol screen.
- **Connector.** Which connector at the rear of the workstation is the valve connected to?
- **Position Names.** These fields let you name each valve position.

**Table 7-1.
Valve Setup Information**

Valve Type	Valve Name/ Function	Position Names	Notes
SVT3-2 Low pressure solenoid valve Note: Indicate the location of the valve cable connection. When the Maximizer is used, inlets A and B become A1, A2, B1, B2	Fraction Collector Diverter	1 Waste 2 Collect	Functions as a fraction collection diverter, determined by the actual fraction collection parameters chosen.
	Inlet A	Named by user	When used before the inlet to Pump A or B, the valve enables buffer selection. The buffer name specified for each position will appear in the Protocol screen's "Isocratic Flow", "Linear Gradient", and "Change Valve" dialog box. Refer to Chapter 8, Sample Loading, for examples.
	Inlet B	Named by user	
	Aux Pump Inlet	Named by user	Used for auxiliary pump load selection to select one of two solutions. Refer to Chapter 8, Sample Loading, for examples.
	User Assigned Name	Named by user	When used for a purpose other than described above. The name specified for each position will appear in the Protocol screen's "Change Valve" dialog box.
SV5-4 Low pressure solenoid valve Note: Indicate the location of the valve cable connection. When the Maximizer is used, inlets A and B become A1, A2, B1, B2	Inlet A	Named by user	When used before the inlet to Pump A or B, the valve provides preparative sample loading or buffer selection. The buffer or sample name specified for each position will appear in the Protocol screen's "Isocratic Flow", "Linear Gradient", and "Change Valve" dialog box. Refer to Chapter 8, Sample Loading, for examples.
	Inlet B	Named by user	
	Aux Pump Inlet	Named by user	Used for auxiliary pump load selection to select one of four solutions. Refer to Chapter 8, Sample Loading, for examples.
	User Assigned Name	Named by user	When used for a purpose other than that described above. The name specified for each position will appear in the Protocol screen's "Change Valve" dialog box.
AVR7-3 High pressure valve Note: Indicate the location of the valve cable connection	Sample Inject	1 Load Sample 2 Inject Sample 3 Purge	For automatically loading a sample.
	User Assigned Name	Named by user	When used for a purpose other than described above. The name specified for each position will appear in the Protocol screen's "Change Valve" dialog box. Refer to Section 4, Advanced System Applications, Chapters 8 through 10.

Table 7-1. (continued)
Valve Setup Information

Valve Type	Valve Name/ Function	Position Names	Notes
AVR9-8 High pressure valve Note: Indicate the location of the valve cable connection. When the Maximizer is used, inlets A and B become A1, A2, B1, B2.	Aux Pump Inlet	Named by user	Used for auxiliary pump load selection to select from up to eight samples, buffers, or rinse solution.
	Inlet A	Named by user	When used before the inlet to Pump A or B, the valve provides preparative sample loading or buffer selection. The buffer or sample name specified for each position will appear in the Protocol screen's "Isocratic Flow", "Linear Gradient", and "Change Valve" dialog box. Refer to Section 4, Advanced System Applications, Chapters 8 and 9.
	Inlet B	Named by user	
	Fraction Collector	1 Waste 2 Collect 3-8 Named by user	Useful for collecting large volume samples. Collects up to 8 samples. The name specified for each position will appear in the Protocol screen's "Change Valve" dialog box.
	User Assigned Name	Named by user	When used for purposes other than described above. The name specified for each position will appear in the Protocol screen's "Change Valve" dialog box. Refer to Chapters 8 and 9.
	Column Switching	Named by user	Allows you to install up to 8 columns and assign valve numbers and names to each column. Requires two AVR9-8 valves: one is an inlet valve and the other functions as an outlet valve.

7.3 PROTOCOL SCREEN

The Protocol screen allows you to program step-by-step instructions for running a method.

Note: Methods may be programmed either by Volume (ml) or Time (min). The default (time or volume) is set by selecting **Edit User Preferences** from the Options drop-down menu.

The Protocol screen contains the following types of icon buttons, which are shown in Figure 7-4 and described in detail in this section.

- **Add Step buttons:** These buttons are used to define the steps in the protocol. They are located in a vertical box on the left side of the screen. To insert a step, highlight the step *below* where the new step is to be added, and then press the appropriate button to begin defining that step. For example, selecting Isocratic Flow displays a dialog box allowing you to define the buffer and volume (or time) and flow rate.
- **Fraction Collector button:** This button is used to define the fraction collection scheme. It is located below the Add Step buttons.
- **Edit buttons:** These buttons, located in the Toolbar, are used to edit, cut, copy, paste, and delete steps from the method. To cut, copy, or delete a step, select that step and then press the appropriate button. To paste, select the step below where the step is to be added and then paste.
- **File Buttons:** Use the New Method, Edit Method, and Browser Toolbar buttons to create a new method or to open an existing method.

The system checks the setup information before allowing you to add steps or run the method. This ensures that each device you need is present and available for the required function.

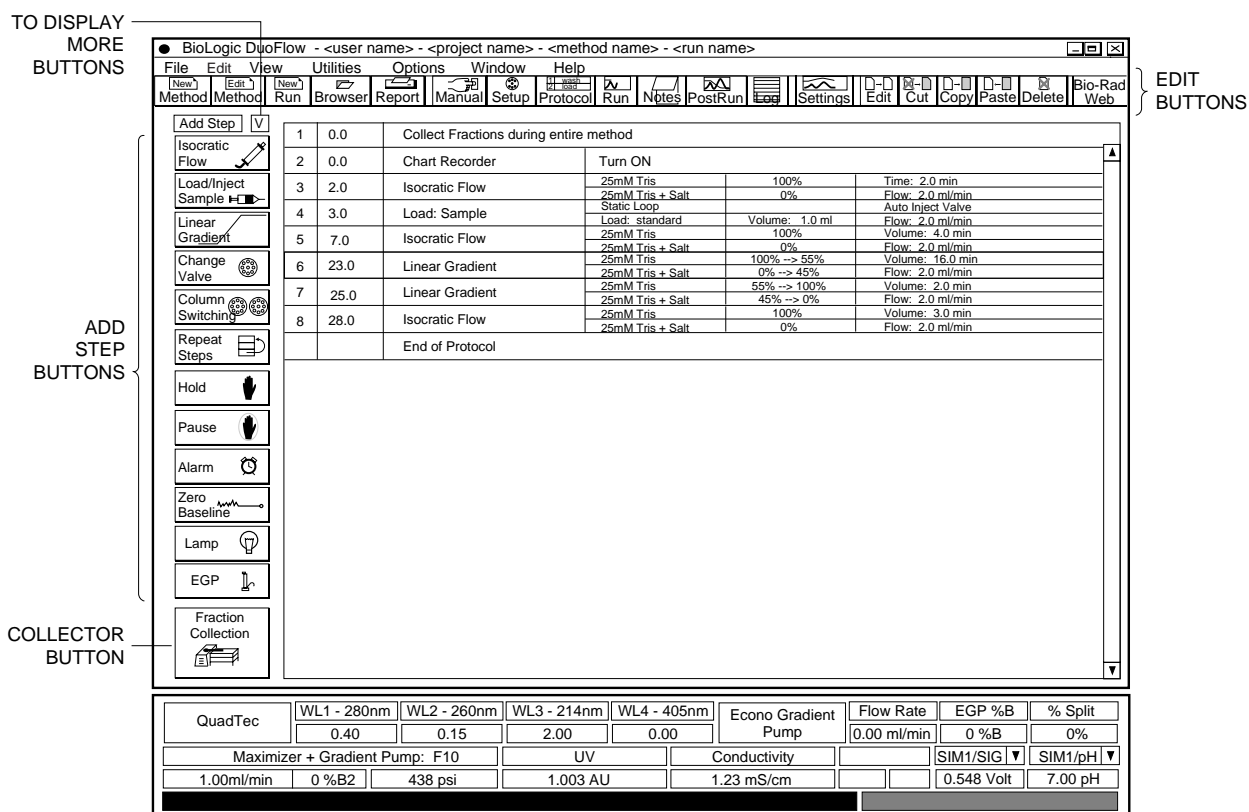


Figure 7-4. Protocol Screen

Table 7-2.
Isocratic Flow

Edit Isocratic Flow

Isocratic Flow

Buffers % Composition

Buffer A1 ▼ A 100 ▲

Buffer B1 ▼ B 0 ▲

Volume (ml.)

1.0 ▲▼

Flow (ml./min.)

1.00 ▲▼

Step 2, Volume = 0.00 ml OK Cancel

Isocratic Flow Screen

Edit Isocratic Flow pH View

Isocratic Flow pH View

Buffer System: Tris (25 mM)
pH Range = 7.20 to 9.20

Solution A1: Tris.HCl (50 mM)
Solution A2: Tris (50 mM)
Solution B1: Degassed Water
Solution B2: Sodium Chloride (2.00M)

Molarity at 100%B = 1.0 M

pH

7.00

%B

0

Volume (ml.)

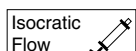
1.0

Flow (ml./min.)

1.00

Step 2, Volume = 1.00 ml OK Cancel

Isocratic Flow Screen with Maximizer in Use



The **Edit Isocratic Flow** screen is used to deliver a buffer under isocratic conditions. The composition may be chosen by changing either Buffer A or Buffer B percent values which increase or decrease in tandem. Use the up/down arrows or input values from the keyboard when the % Composition field is highlighted. The following selections are available in this screen.

- **Buffers:** Displays all available buffers for separation. Buffer A and Buffer B can be labeled to identify the buffer in use by editing the Gradient Pump fields in the Setup screen. If pump inlet valves are assigned in the Setup screen and buffers are defined, all additional buffers will be displayed in the drop-down boxes.
- **Composition:** Allows you to choose the buffer composition (e.g., 75% A and 25% B).

The **Edit Isocratic Flow pH View** screen is used to deliver a buffer under isocratic conditions with a Maximizer connected to your Workstation. The pH and salt concentration (%B) may be set by using the up/down arrows or by inputting values from the keyboard when this field is highlighted. The dialog box on the left-hand side lists the current buffer system and its pH range as well as the solutions present at each Maximizer valve inlet. The following selections are available in this screen.

- **pH:** Allows you to enter the pH for the current step.
- **%B:** Allows you to enter the salt concentration as a percentage of the maximum allowable concentration.

The following selections are available in both the **Edit Isocratic Flow** screen and the **Edit Isocratic Flow pH View** screen.

- **Volume/Time:** Allows you to choose the duration of the step.
- **Flow rate (ml/min):** Allows you to choose the flow rate of the step.
- **OK:** Adds the step to the protocol. This is the same as pressing the **Enter** key on the keyboard.
- **Cancel:** Cancels all input. This is the same as pressing the **Esc** key on the keyboard.
- **Step, Time or Volume:** Identifies current step number, and calculates the elapsed time or volume from all previous steps. This is not user editable.

Table 7-3.
Load/Inject Sample

Edit Load/Inject Sample

Load/Inject Sample Fill Before Inject Rinse After Inject

Type

☒ Static Loop

☐ Dynamic Loop

☐ Direct Inject

Injection Buffers

Buffer A1 A 100

Buffer B1 B 0

% Comp.

Volume (ml.) 1.0

Flow (ml./min) 1.00

Auto Inject Valve will move to INJECT at start of step and return to LOAD at end of step.

Step 3, Volume = 1.00 ml

OK Cancel

Load/Inject Sample Screen

Edit Load/Inject Sample

Load/Inject Sample Fill Before Inject Rinse After Inject

Type

☒ Static Loop

☐ Dynamic Loop

☐ Direct Inject

Buffer System: Tris (25 mM)
pH Range = 7.20 to 9.20

Solution A1: Tris.HCl (50 mM)

Solution A2: Tris (50 mM)

Solution B1: Degassed Water

Solution B2: Sodium Chloride (2.00M)

Molarity at 100%B = 1.0 M

pH 7.00

%B 0

Volume (ml.) 1.0

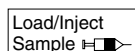
Flow (ml./min) 1.00

Auto Inject Valve will move to INJECT at start of step and return to LOAD at end of step.

Step 2, Volume = 1.00 ml

OK Cancel

**Load/Inject Sample Screen
with a Maximizer in Use**



Use this button to program the sample injection. The **Edit Load/Inject Sample** screen allows you to specify the device that will load sample onto the column, as well as the volume of sample to be loaded. (If programming in time mode, the system calculates the time required for this step.) When the Maximizer is not being used, the **Edit Load/Inject Sample** screen displays the following.

- **Injection Buffers and % Composition:** These fields allow you to choose the buffer and its composition that will push the sample through the sample loop.

When you are using a Maximizer in Buffer Blending mode, the **Edit Load/Inject Sample** screen also displays the following selections.

- **pH:** Allows you to enter the pH for the current step. The dialog box on the left-hand side lists the currently defined buffer system and its pH range as well as the solutions present at each inlet.
- **%B:** Allows you to enter the salt concentration as a percentage of the maximum allowable concentration.

Table 7-3. (continued)
Load/Inject Sample

The following selections are available in both screens.

- **Load/Inject Sample:** Selects injection type. The AVR7-3 sample inject valve will move to the Inject position at the start of the step and return to the Load position at the end of the step.

Static Loop: Standard fixed volume loop for sample loading. Used with the AVR7-3 valve, sample can be loaded with a syringe through port 2, and the valve can rinse the sample. An auxiliary (AUX) pump and valve (such as the SVT3-2, SV5-4, or AVR9-8), may be used to fill the loop on the AVR7-3 prior to injection onto the column. Use **Fill Before Inject** and **Rinse After Inject** to select loop fill volume and flow rate. Refer to Chapter 8 for discussion of how to select volume fill and rinse.

Dynamic Loop: Uses Bio-Rad's DynaLoop or other sliding-piston sample loop. Both partial loop and full loop injections can be programmed. The dynamic loop can be filled manually through Inject valve port 2. An auxiliary (AUX) pump and valve (such as the SVT3-2, SV5-4, or AVR9-8), may be used to fill the loop on the AVR7-3 prior to injection onto the column. Use **Fill Before Inject** to select loop fill volume and flow rate. The Rinse function is not available. See Chapter 8 for DynaLoop loading instructions.

Direct Inject: Allows direct injection of sample through either the Workstation pump or auxiliary load pump. Pre-pump valves (such as the SVT3-2, SV5-4, or AVR9-8) automate direct injection. Use of an auxiliary pump (such as the Bio-Rad Model EP-1 Econo pump or Econo Gradient Pump) allows direct injection of sample onto a low pressure column. Use of an auxiliary (AUX) pump and valve allows multiple direct injections onto a low pressure column. Refer to Chapter 8 for discussion of a direct inject applications.
- **Volume:** Allows you to specify the volume of the sample to be injected. The smallest volume selectable is 0.1 ml; the largest volume selectable is 9999 ml (for larger volumes, use another Load/Inject Sample step). Actual load volume is defined by the sample loop size.
- **Flow Rate:** Allows you to specify the flow rate for the sample injection step.
- **OK:** Adds the step to the protocol. This is the same as pressing the **Enter** key on the keyboard.
- **Cancel:** Does *not* add the step to the protocol. This is the same as pressing the **Esc** key on the keyboard.
- **Step, Time or Volume:** Identifies current step number, and calculates the elapsed time or volume from all previous steps. This is not user editable.

Table 7-4. Linear Gradient

Edit Linear Gradient

Linear Gradient

Buffers	% Composition	Initial	Final	Volume (ml.)	Flow (ml./min)
Buffer A1	A	100	100	1.0	1.00
Buffer B1	B	0	0		

Step 8, Volume = 2.00 ml

OK Cancel

Edit Linear Gradient pH View

Linear Gradient pH View

Buffer System: Tris (25 mM)
pH Range = 7.20 to 9.20

Solution A1: Tris.HCl (50 mM)
Solution A2: Tris (50 mM)
Solution B1: Degassed Water
Solution B2: Sodium Chloride (2.00M)
Molarity at 100%B = 1.0 M

pH: 7.00

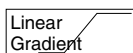
Initial %B: 0 Final %B: 100

Volume (ml.): 1.00
Flow (ml./min): 1.00

Step 2, Volume = 1.00 ml

OK Cancel

Linear Gradient Screen

Linear Gradient Screen
with a Maximizer in
Buffer Blending Mode

Use the **Edit Linear Gradient** screen to deliver buffer gradients. The Linear Gradient screen sets the initial and final composition of the buffers and the period over which the change in composition is to occur. There is no limit to the number of gradient steps in a protocol. The following selections are available.

- **Buffers:** Allows you to choose two buffers to make a binary gradient. Drop-down menus display all buffers that are set up using Workstation pump inlet valves.
- **% Composition:** Allows you to choose initial and final values that will form a linear binary gradient. Notice that changing one value in the "Initial" column affects the other "initial" value. The "Final" column behaves identically.

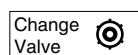
The **Edit Linear Gradient pH View** screen appears when you are using a Maximizer in Buffer Blending mode. This box sets the pH, initial and final salt concentration, and the period over which the change in composition is to occur. There is no limit to the number of gradient steps in a protocol. The following selections are available.

- **pH:** Allows you to enter the pH for the current step. The dialog box on the left-hand side lists the currently defined buffer system and its pH range as well as the solutions present at each inlet.
- **Initial %B and Final %B:** Allows you to set the initial and final salt concentration as a percentage of the maximum allowable concentration for a linear binary gradient.

The following selections are available in both screens.

- **Volume:** Allows you to choose the duration of this step.
- **Flow:** Allows you to choose the flow rate of this step.
- **OK:** Adds the step to the protocol. This is the same as pressing the Enter key on the keyboard.
- **Cancel:** Does not add the step to the protocol. This is the same as pressing the Esc key.
- **Step, Time or Volume:** Identifies current step number, and calculates the elapsed time or volume from all previous steps. This is not user editable.

Table 7-5.
Change Valve



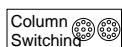
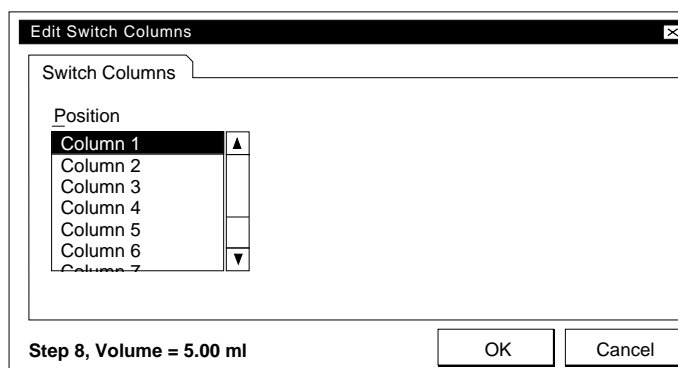
To select any valve and change its position.

- **Change Valve name and position:** These fields allow you to choose a valve and to make a change in valve position. Note that certain valve functions defined in the Setup screen (such as Fraction Collector Diverter, Aux Pump Inlet, Sample Inject, Inlet A, Inlet B) are “tied” to other protocol steps, so valve position changes will be made automatically. An example would be an AVR7-3 valve defined as a Sample Inject valve in the Setup screen, which is then tied to the Load/Inject Sample step in the Protocol screen.

Valves assigned a “User-defined name” during Setup require the “Change Valve” step at the desired point in the protocol.

- **OK:** Adds the step to the protocol. This is the same as pressing the **Enter** key on the keyboard.
- **Cancel:** Does **not** add the step to the protocol. This is the same as pressing the **Esc** key on the keyboard.
- **Step, Time or Volume:** Identifies current step number, and calculates the elapsed time or volume from all previous steps. This is not user editable.

Table 7-6.
Column Switching

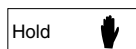


The DuoFlow system supports the use of more than one column during a run. (Chapter 9 provides two examples of column switching during a run.) Use this button to specify which column to load sample onto.

Column switching is supported by installing two AVR9-8 valves that permit the use of 8 different analytical and separation columns. The AVR9-8 valves are defined by selecting Column Switching in the Setup screen. This identifies the two valves as installed and synchronizes the switching of the valves without further user input. Column switching permits identification of the electrical connection port of each valve and user naming for each column. Installation of the AVR9-8 valve in setup automatically activates Column Switching in the Protocol screen.

- **Position:** Select the column to switch to in a run.
- **OK:** Adds the step to the protocol. This is the same as pressing the **Enter** key on the keyboard.
- **Cancel:** Does *not* add the step to the protocol. This is the same as pressing the **Esc** key on the keyboard.
- **Step, Time or Volume:** Identifies current step number, and calculates the elapsed time or volume from all previous steps. This is not user editable.

Table 7-7.
Hold



Inserts a Hold step into the method. The Hold step stops the progression of the method; the pumps continue pumping at the Hold step %B composition. The method time (or volume) advances. When the run resumes, the current fraction collection condition is maintained. The hold condition is maintained until a specified activity occurs (e.g., “Hold until Start of Inject” or “Hold until Keypress”). For example, if the hold starts at time 2 (minutes), then the method resumes at time 2, no matter how long the Hold was in effect.

Hold Until and Threshold Detector: Choose one of five events to discontinue the hold:

- **Key Pressed:** Press the **F2** key on the keyboard to end the programmed hold.
- **Start of Inject:** The run will continue when the manually-controlled device (which must be connected to the Workstation AUX connector at pin 1, Inject) is moved to its desired position.
- **End of Inject:** The run will continue when the manually-controlled device (which must be connected to the Workstation AUX connector at pin 1, Inject) is returned to its original position.
- **Above Threshold:** The run will continue when threshold exceeds that specified by the following fields in this window:

Threshold Detector: Select the desired threshold detector absorbance, conductivity, RI, etc.

Threshold: Specify a threshold value.

- **Below Threshold:** The run will continue when threshold falls below that specified by the following fields in this window:

Threshold Detector: Select the desired threshold detector absorbance, conductivity, RI, etc.

Threshold: Specify a threshold value.

- **Time Out Req'd and Time Out (min):** To hold for a specified length of time.
- **Sound Alarm:** When this box is checked, an alarm will sound at the beginning of the step to remind you that the system requires an action on your part to allow the method to advance.
- **OK:** Adds the step to the protocol. This is the same as pressing the **Enter** key on the keyboard.
- **Cancel:** Does *not* add the step to the protocol. This is the same as pressing the **Esc** key on the keyboard.
- **Step, Time or Volume:** Identifies current step number, and calculates the elapsed time or volume from all previous steps. This is not user editable.

Table 7-8.
Miscellaneous

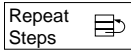
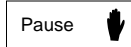


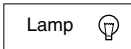
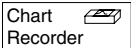
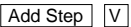

Button	Description
	To repeat the highlighted step(s) a specified number of times. To highlight more than one step, hold down the Ctrl key while selecting steps with the mouse.
	To pause the method at a specific step during the run. Pause stops the progression of the method and time, holds the %B composition, and stops the pumps. Time Out Req'd permits a pause time to be entered. The step will be paused for the specified length of time, after which it will automatically resume. An audible alarm may be programmed to sound when the step is paused and when it automatically resumes.
	To sound a 10 second audible alarm at the programmed time. The progress of the run will not be affected.
	To zero the detector baseline at a programmed time.
	At system start-up, the default condition is Lamp ON. The lamp may be set to turn off at the end of a run. If the lamp is OFF, it takes about 20 minutes for the lamp to warm up prior to use.
	To turn the chart recorder on/off at the programmed time. Inserts a step that tells the chart recorder to start/stop the paper feed and lower/lift the pen.
	To display additional steps so that they can be added to the method.

Table 7-9.
Fraction Collection

Button	Description: Collect All
	To set up fraction collection. Fraction collection schemes are discussed below.

IMPORTANT NOTE:

The DuoFlow system is designed to control the BioFrac and the Model 2128 fraction collectors, which are the only collectors that provide a choice of racks and the ability to overlay fractions from consecutive runs.

The DuoFlow system will also accommodate the Bio-Rad Model 2110 fraction collector as well as generic collectors. To use a fraction collection scheme other than “Collect All” with these collectors, it is **essential** to assign a SVT3-2 valve as a “Fraction Collector Diverter” in the Setup screen. Rack choice and overlay features are **not** available with these collectors.

Fraction Collection Scheme

BioFrac Rack Type: F1 (12-13 mm tubes)

☒ Collect All

☐ Threshold

☐ Collection Windows

☐ Threshold and Collection Windows

Threshold Detector

UV Detector

Threshold

0.100 AU

Close

Fraction Size

1.00 ml

Start Tube

1

End Tube

180

Non-peak Parameters:

Destination:

☒ Waste

☐ Tubes

Fraction Size

1.00 ml

Tubes Required

180

Number of Racks

1

Collect All: The eluant from the entire run will be collected by the fraction collector.

- **Fraction Size:** Enter the fraction size as volume (ml).
- **Tubes Required** and **Number of Racks:** The displayed value is calculated based on the total volume divided by fraction size.
- **Start Tube** and **End Tube:** This applies only to the BioFrac and the Model 2128 fraction collector. Identify the first and last tubes to receive fractions.

Table 7-9. (continued)
Fraction Collection

Description: Threshold

The screenshot shows the 'Fraction Collection Scheme' dialog box. On the left, under 'BioFrac Rack Type: F1 (12-13 mm tubes)', there are four radio button options: 'Collect All', 'Threshold' (which is selected), 'Collection Windows', and 'Threshold and Collection Windows'. The main area is divided into two sections. The top section, 'Threshold Detector', has a dropdown menu set to 'UV Detector'. To its right, the 'Threshold' is set to '0.100' with up/down arrows and the unit 'AU'. A 'Close' button is in the top right. The bottom section, 'Non-peak Parameters:', has a 'Destination:' label with two radio button options: 'Waste' (selected) and 'Tubes'. To the right of this is a 'Fraction Size' input set to '1.00' with up/down arrows and the unit 'ml'. In the middle of the dialog, there are three input fields with up/down arrows: 'Fraction Size' set to '1.00' ml, 'Start Tube' set to '1', and 'End Tube' set to '180'.

Threshold: This method allows the flow to be sent to the collector if the detector signal of the eluant is above a certain signal level. **Note:** You can select any detection source to initiate threshold-based fraction collection. Non-peak eluant can be sent to waste or to the fraction collector.

- **Fraction Size, Start Tube, and End Tube:** See description for **Collect All** on page 7-16.
- **Threshold:** Enter the threshold value in absorbance units. Fractions will be collected whenever the detector output goes above the threshold level.
- **Non-peak parameters Destination and Fraction Size:** Enter the non-peak parameters. If the buffer stream is diverted to Waste, there is no need to enter a fraction size. But if you want to collect non-peak material, you must enter a fraction size.

Table 7-9. (continued)
Fraction Collection

Description: Collection Windows

Fraction Collection Scheme
 BioFrac Rack Type: F1 (12-13 mm tubes)
☐ Collect All
☐ Threshold
☒ **Collection Windows**
☐ Threshold and Collection Windows

Threshold Detector
 UV Detector

Threshold
 0.100

Fraction Size
 1.00 ml

Start Tube
 1

End Tube
 180

Tubes Required
 Number of Racks
 180
 1

Non-peak Parameters:
 Destination:
☒ Waste
☐ Tubes

Fraction Size
 1.00 ml

Close

Fraction Collector Scheme
CollectionWindows

	Start (ml)	End (ml)	Frac. Size (ml)			
	0.0	4.0	0.60			
1	4.0	5.0	0.60			
2	7.0	6.0	1.00			

Close
 ADD MODE
 Save Window
 Delete Window
 Finished Adding

Fraction Collector Scheme
CollectionWindows

	Start (ml)	End (ml)	Frac. Size (ml)	Thresh. (AU)	Non-Peak Frac. Size (ml)	
1	0.0	4.0	1.00	1.00	<input checked="" type="radio"/> Waste <input type="radio"/> Tubes	0.01
2	4.0	5.0	0.60	0.000	Waste	
3	7.0	6.0	1.00	0.010	Waste	

Close
 EDIT MODE
 Add Window
 Delete Window
 Cancel Editing

Collection Windows: This method specifies collection during specified parts of the run (Time or Volume). Each collection window can have a unique fraction size (ml).

Note: Collection windows programming should be done after all other steps have been written. When using Collection Windows, the BioFrac and Model 2128 will skip a tube between each window collection. For example, if the first window ends collection at 20 minutes and the second window starts collection starting at 21 minutes, then the fraction collector will leave an empty tube for the period between minutes 20 and 21.

- **Fraction Size, Tubes Required, Number of Tubes, Start Tube, and End Tube:** See description for **Collect All** on page 7-16.
- **Add Mode:** Enter the desired values for **Start**, **End**, and **Frac Size**.
 - Select the **Save Window** button and the values will appear in line 1.
 - Enter the values for the next window and select **Save Window**. The values will appear in line 2.
 - When the collection scheme is complete, select **Finished Adding**, and the Select mode will appear.
- **Select Mode:** This mode (not shown in illustration above) shows the following two buttons:
Add Window: Inserts a window after the highlighted window.
Delete Window: Deletes that window.

Table 7-9. (continued)
Fraction Collection

Description: Threshold and Collection Windows

Fraction Collection Scheme

BioFrac Rack Type: F1 (12-13 mm tubes)
☐ Collect All
☐ Threshold
☐ Collection Windows
☒ Threshold and Collection Windows

Threshold Detector
 UV Detector

Threshold
 0.100 AU

Fraction Size
 1.00 ml

Start Tube
 1

End Tube
 180

Non-peak Parameters:
 Destination:
☒ Waste
☐ Tubes

Fraction Size
 1.00 ml

Close

Fraction Collector Scheme

CollectionWindows

Start (ml)
0.0

End (ml)
5.0

Frac. Size (ml)
1.00

Thresh (AU)
1.00

Non-Peak Frac. Size (ml)
0.01

☒ Waste
☐ Tubes

	Start (ml)	End (ml)	Frac. Size (ml)	Thresh (AU)	Non-Peak Frac. Size (ml)	
1	4.0	5.0	0.60	0.000	Waste	
2	7.0	6.0	1.00	0.010	Waste	

Close

ADD MODE

Save Window

Delete Window

Finished Adding

Fraction Collector Scheme

CollectionWindows

Start (ml)
0.0

End (ml)
5.0

Frac. Size (ml)
1.00

Thresh. (AU)
1.00

Non-Peak Frac. Size (ml)
0.01

☒ Waste
☐ Tubes

	Start (ml)	End (ml)	Frac. Size (ml)	Thresh. (AU)	Non-Peak Frac. Size (ml)	
1	4.0	5.0	0.60	0.000	Waste	
2	7.0	6.0	1.00	0.010	Waste	

Close

SELECT MODE

Add Window

Delete Window

Fraction Collector Scheme

CollectionWindows

Start (ml)
0.0

End (ml)
5.0

Frac. Size (ml)
1.00

Thresh. (AU)
1.00

Non-Peak Frac. Size (ml)
0.01

☒ Waste
☐ Tubes

	Start (ml)	End (ml)	Frac. Size (ml)	Thresh. (AU)	Non-Peak Frac. Size (ml)	
1	0.0	5.0	1.00	1.00	Waste	
2	4.0	5.0	0.60	0.000	Waste	
3	7.0	6.0	1.00	0.010	Waste	

Close

EDIT MODE

Add Window

Delete Window

Cancel Editing

Threshold and Collection Windows: Each Collection Window (discussed on page 7-18) can also contain a threshold value to further discriminate when eluant can be collected. For each collection window, non-peak eluent can be directed to waste or to the fraction collector. The Threshold and Time windows can be combined, so the system collects fractions only during the programmed Time windows and within the Time windows only when UV absorbance is above the threshold value.

When using the Collection Windows functions, the BioFrac and Model 2128 will skip a tube between each window collection. For example, if the first window ends collection at 20 minutes and the second window starts collection starting at 21 minutes, then the fraction collector will leave an empty tube for the period between minutes 20 and 21.


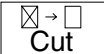
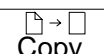
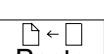
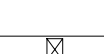
Table 7-9. (continued)
Fraction Collection

Description: Threshold and Collection Windows (continued)

- **Fraction Size, Start Tube, and End Tube:** See description for **Collect All**.
 - **Start (ml), End (ml), Fraction Size (ml), Thresh (AU), and Non-Peak Frac Size (ml):** Each window is defined by these parameters. Use the scroll bar to the right of the window display to scroll through the list of windows. Enter the fraction size as volume (ml).
 - **Add Mode:** Enter the desired values for **Start, End, Frac Size, and Thresh**.
Select the **Save Window** button and the values will appear in line 1.
To enter the values for the next window, select **Add Window** and enter the values. Select **Save Window** and the values will appear in line 2.
When the collection scheme is complete, select **Finished Adding**, and the Select mode will appear.
 - **Select Mode:** This mode shows the following two buttons:
Add Window: Inserts a window after the highlighted window.
Delete Window: Deletes that window.
 - **Non-peak parameters Destination and Fraction Size:** Enter the non-peak parameters. When collecting non-peak material, you must enter a fraction size.
-

The following Toolbar menu options are available only when creating and editing a protocol.

Table 7-10.
Protocol Screen's Editing Toolbar

Button	Description
Edit buttons:	These buttons are available from the System menu.
 Edit	To edit the currently highlighted step in the protocol.
 Cut	To cut the currently highlighted step from the protocol. It places the step in the clipboard, which means it can be placed elsewhere by using the Paste button.
 Copy	To copy the currently highlighted step in the protocol.
 Paste	To paste the cut or copied step before the currently highlighted step.
 Delete	To delete the currently highlighted step from the protocol.

7.4 RUN SCREEN

The Run screen displays a run in progress. All data associated with the run are automatically saved. An example of a run in progress is shown in Figure 7-5 below. Table 7-11 discusses the buttons which may be used to control the run. In addition, if the EZLogic Integration software is installed, the **Integ.** toolbar button replaces the **Log** button.

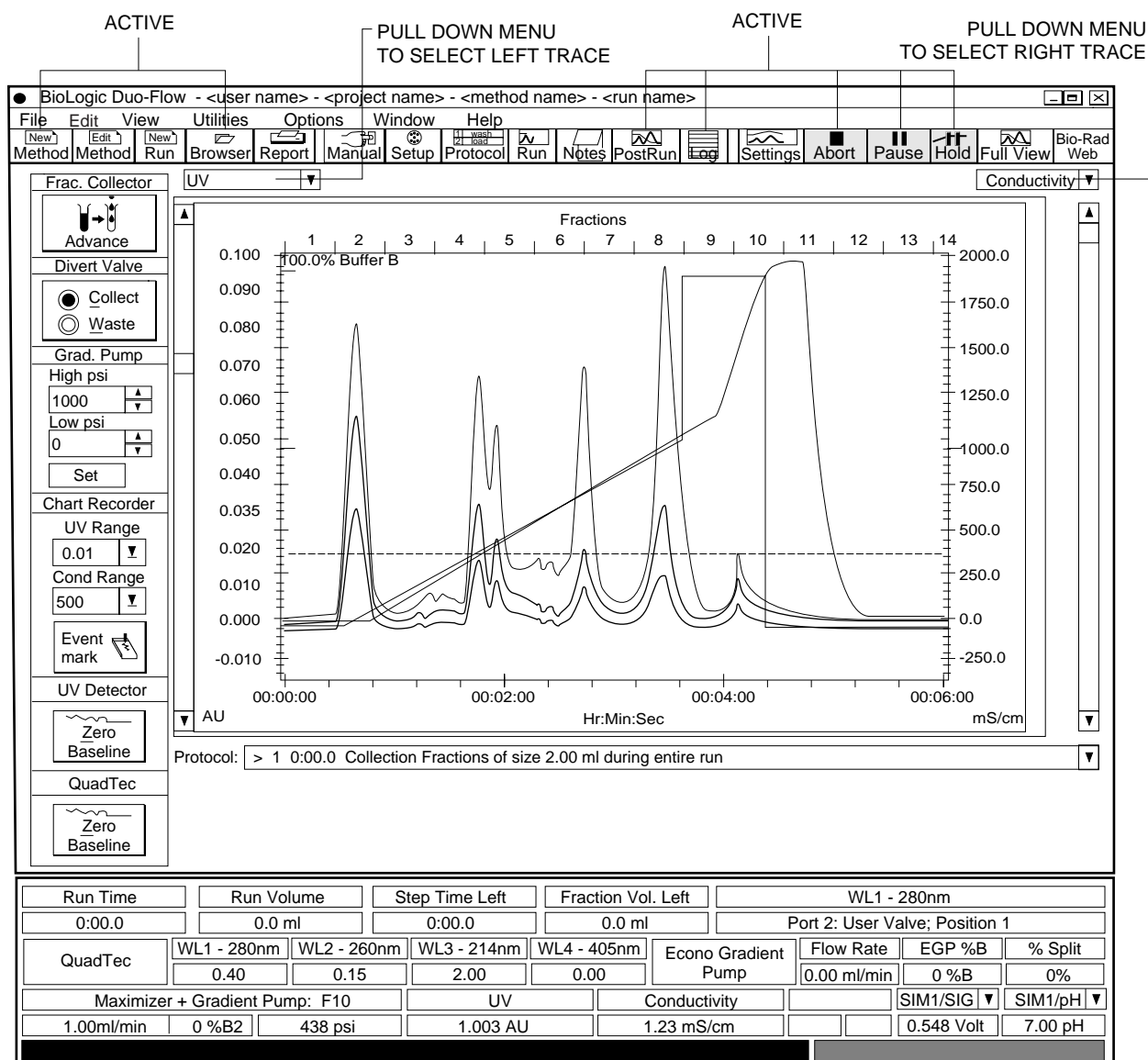


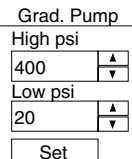
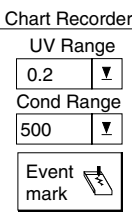
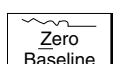
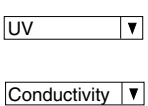
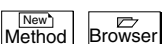





Figure 7-5. Run Screen showing a Run in Progress

Table 7-11.
Run Screen's Control

Button	Description
	Advances the fraction collector to the next tube. Pressing this button does not modify the method. The event is recorded in the Run Log.
	Immediately changes the position of the diverter valve. Pressing this button does not modify the method. The event is recorded in the Run Log.
	To set the pressure limits of the Workstation pumps. This is not part of a protocol; it is a system setting from the Manual screen. The method pauses and the pumps stop when the pressure goes above or below the set values.
	<p>To set the UV Detector range for the chart recorder output. The settings are: 0.001, 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0 AUFS.</p> <p>To set the Conductivity range on the chart recorder output. The settings are: 500, 200, 100, 50, 20, 10, 5 mS/cm.</p> <p>To set an Event Mark on the chart recorder UV trace output only. It will not record event marks on the screen.</p>
	<p>Use the Zero Baseline button to reset the UV or QuadTec detector absorbance value to zero.</p> <p>Note: Use care during a collection scheme which uses a Threshold value.</p>
	These drop-down menus, located on either side of the chromatogram, are used to select the active data trace to be scaled during or after a run. As each trace scale is selected, the scroll bars can be used to adjust the scale setting.
	To work offline while a run is in progress. (Refer to section 7.4.2, Working Offline.)
	<p>Notes displays the Run Notebook screen, which allows you to enter notes regarding the run.</p> <p>Log displays the Run Log screen, which provides information about the run and is non-editable.</p>
	To stop, pause, or hold a method in progress. (Refer to Figure 7-6.)
	To select which traces to display in the chromatograms. (Refer to Table 5-7.)

7.4.1 Pausing/Stopping a Method in Progress

The Run screen provides three options for pausing or stopping a method in progress, as discussed in the figure below.

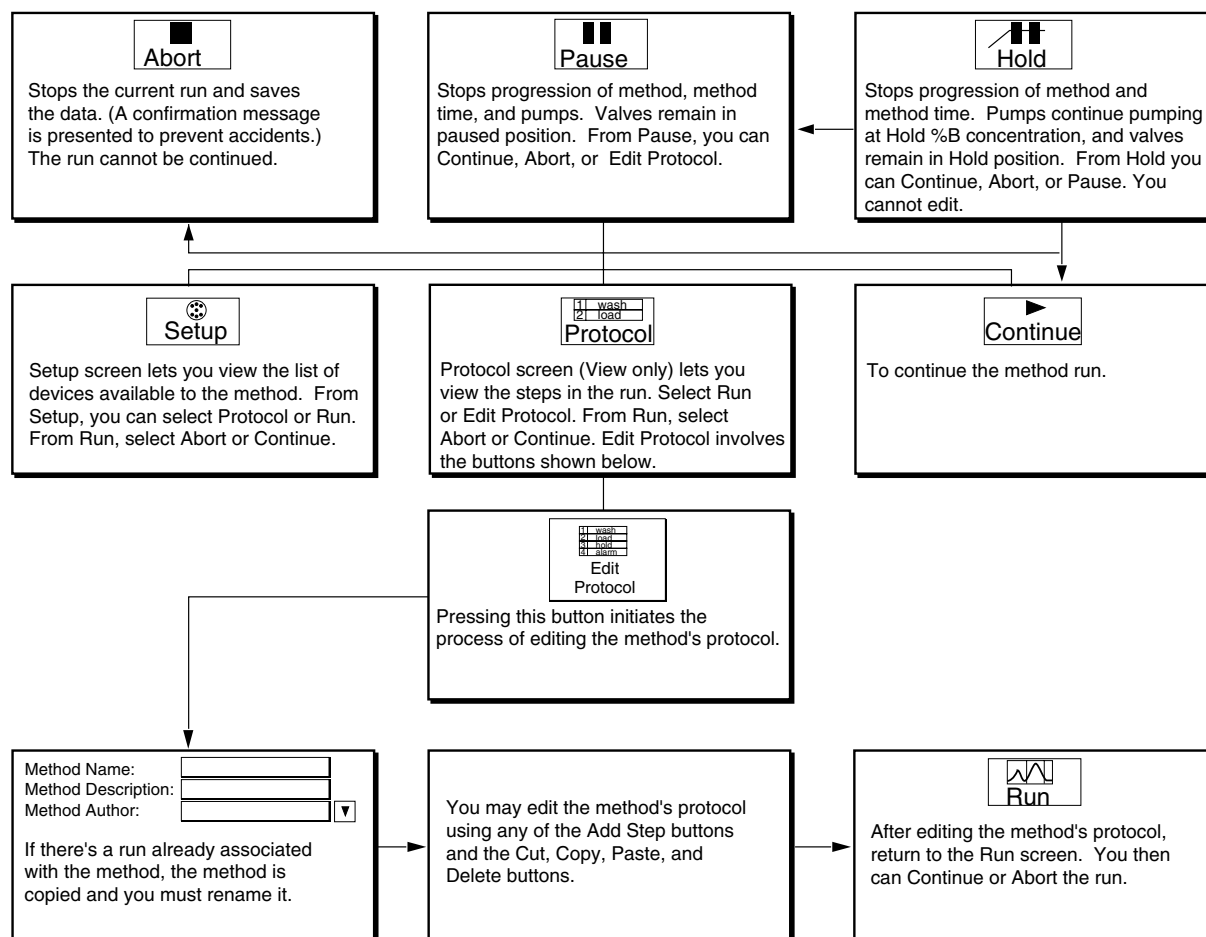


Figure 7-6. Run Screen's Abort, Pause, and Hold Buttons

7.4.2 Working Offline During a Run

The DuoFlow system software allows you to work offline, creating a new method or editing an existing method, while a run is in progress. The following actions can be performed:

- Write a new method
- Edit an existing method
- View results from a completed run
- Print a report from a completed run
- Integrate run data from a completed run (using the optional EZLogic software)
- Perform PostRun analysis on a completed run
- Export Data or Export Chromatogram Image of a completed run
- Access the HELP screen

These functions are not available while a run is in progress:

- PostRun analysis of the run in progress
- Integration of the data from the run in progress
- Copy Out data
- Copy In data
- Initiation of a new run
- Utilities functions, including calibrating pH probe, gradient pumps, conductivity
- Manual mode functions

During a run, the following toolbar buttons activate the offline window:

- **Browser** button

The online and offline windows are distinguished as follows:

- Online: The BioLogic DuoFlow icon in the upper left of the screen is green, and the title bar does not indicate online.
- Offline: The BioLogic DuoFlow icon in the upper left of the screen is yellow, and the title bar indicates offline.

To change between the online and offline windows, you can use any of the following:

- Windows drop-down menu: The online and offline windows are listed.
- Task bar: The Windows® taskbar lists the BioLogic DuoFlow windows.
- Alt-Tab: Simultaneously hold down these two keys to list the open windows.

7.4.3 Editing A Method During a Run

During a run, you may find you want to change some of the run parameters. The figure below shows how this is handled by the system and some of the restrictions.

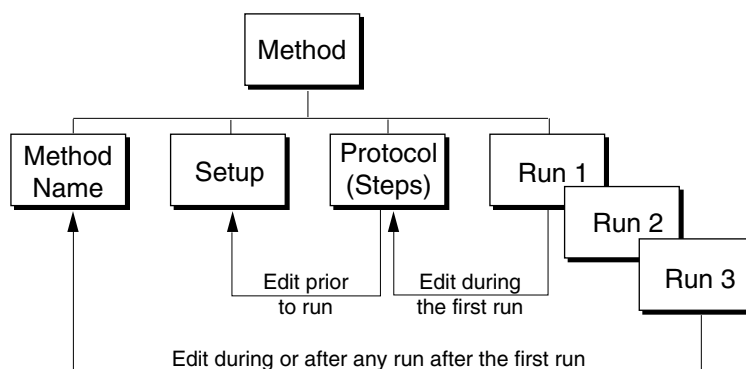


Figure 7-7. Editing during a Run

- During the first run of a method, you can **pause** the method and return to the Protocol screen to edit steps that have not already been started. The method name will not change.
- Any run after the first can be paused and then edited, except runs in queue which cannot be edited. You will first be asked to rename the method (or accept the default new name) when the **Edit Protocol** button is clicked. This is to ensure the integrity of the database in terms of which method protocol particular runs are tied to.

For example, assume you make three runs under a method named “Ion-exchange UNO Q1.” During the fourth run, you may decide to pause the run, and enter the Protocol screen to increase the length of the salt gradient. In this case, you will first be prompted to rename the method (e.g., Ion-exchange UNO Q1 Rev. 1) before proceeding with the edit.

Upon completion of the run, the database of methods and runs will show three runs associated with the method “Ion-exchange UNO Q1” and one run associated with the method “Ion-exchange UNO Q1 Rev. 1.”

- A fraction collection scheme is editable, but it cannot be added to a protocol once a run has been started. In such cases, you should abort the run and select **Edit the Method**.
- A currently programmed fraction collection scheme may be edited during a run, but note that changing the fraction size of a “Collect All” scheme will first require selection of “Collection Windows.” This is because the editing process effectively turns the initial “Collect All” scheme into the “first” collection window.

BioLogic Duo-Flow - offline

File Edit View Utilities Options Window Help

Method Method Run Browser Report Manual Setup Protocol Run Notes PostRun Log Settings Edit Cut Copy Paste Delete Bio-Rad Web

Add Step:

Edit Protocol

View-Only Mode

Fraction Collection

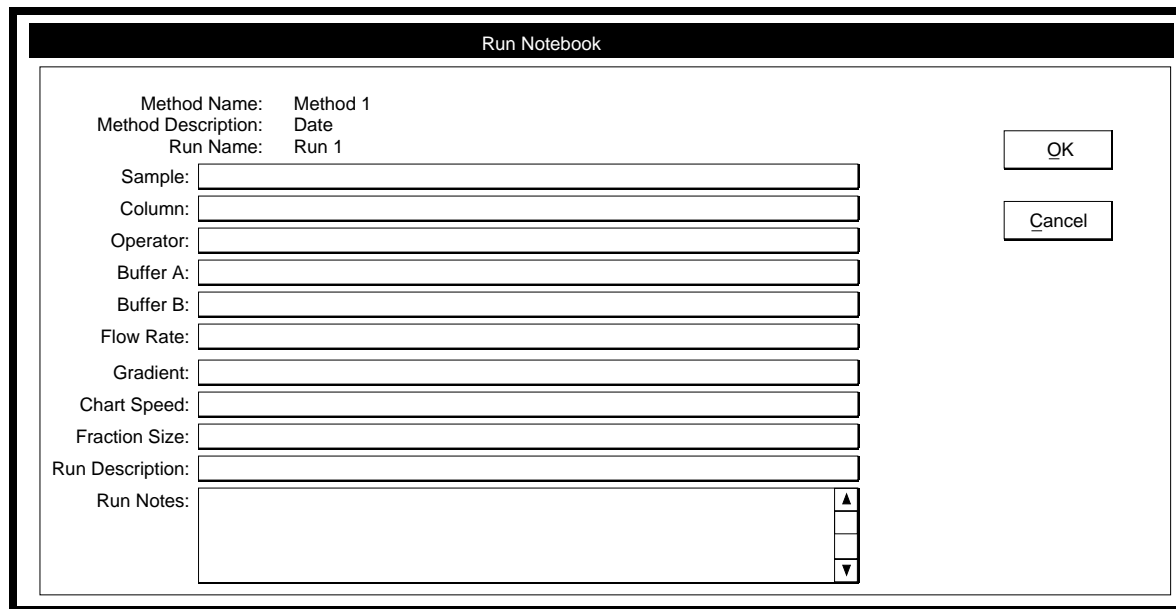
1	0.0	Collect Fractions during entire method		
2	0.0	Chart Recorder	Turn ON	
3	2.0	Isocratic Flow	25mM Tris	100% Time: 2.0 min
			25mM Tris + Salt	0% Flow: 2.0 ml/min
4	3.0	Load: Sample	Static Loop	Auto Inject Valve
			Load, standard	Volume: 1.0 ml Flow: 2.0 ml/min
5	7.0	Isocratic Flow	25mM Tris	100% Volume: 4.0 min
			25mM Tris + Salt	0% Flow: 2.0 ml/min
6	23.0	Linear Gradient	25mM Tris	100% --> 55% Volume: 16.0 min
			25mM Tris + Salt	0% --> 45% Flow: 2.0 ml/min
7	25.0	Linear Gradient	25mM Tris	55% --> 100% Volume: 2.0 min
			25mM Tris + Salt	45% --> 0% Flow: 2.0 ml/min
8	28.0	Isocratic Flow	25mM Tris	100% Volume: 3.0 min
			25mM Tris + Salt	0% Flow: 2.0 ml/min
		End of Protocol		

QuadTec	WL1 - 280nm	WL2 - 260nm	WL3 - 214nm	WL4 - 405nm	Econo Gradient Pump	Flow Rate	EGP %B	% Split
	0.40	0.15	2.00	0.00		0.00 ml/min	0 %B	0%
Maximizer + Gradient Pump: F10			UV	Conductivity		SIM1/SIG ▼		SIM1/pH ▼
1.00ml/min	0 %B2	438 psi	1.003 AU	1.23 mS/cm		0.548 Volt	7.00 pH	

Figure 7-8. Protocol Screen during a Run

7.4.4 Run Notebook Screen

Use the Run Notebook screen to maintain any information you want regarding the run. It contains fields for description of the sample, the column, the operator, the buffer(s), flow rates, gradients, chart speed, fraction size, and any other information you might want to enter.



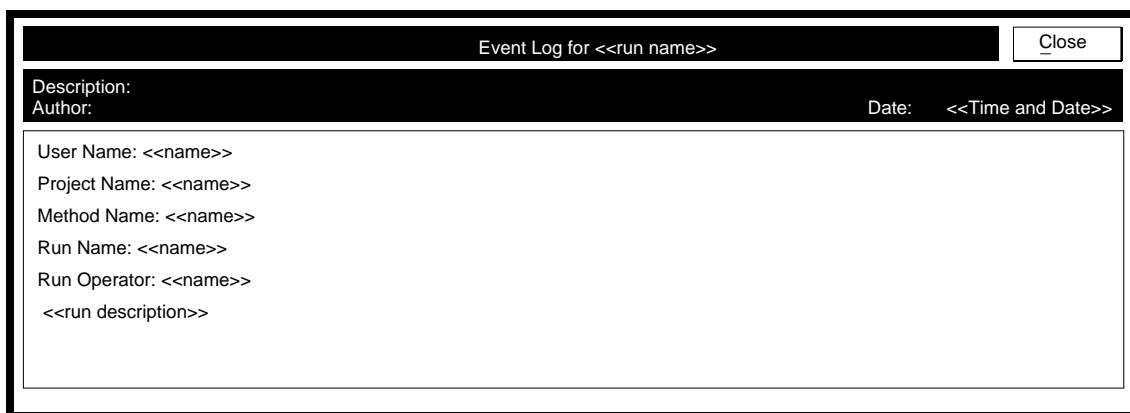
The Run Notebook screen is a dialog box titled "Run Notebook". It contains the following fields and controls:

- Method Name: Method 1
- Method Description: Date
- Run Name: Run 1
- Sample: [Text Field]
- Column: [Text Field]
- Operator: [Text Field]
- Buffer A: [Text Field]
- Buffer B: [Text Field]
- Flow Rate: [Text Field]
- Gradient: [Text Field]
- Chart Speed: [Text Field]
- Fraction Size: [Text Field]
- Run Description: [Text Field]
- Run Notes: [Text Area with Up/Down Arrow Buttons]
- OK button
- Cancel button

Figure 7-9. Run Notebook Screen

7.4.5 Run Log Screen

The Run Log screen details the time and order of the execution of each step, error, and/or event of the run. This information cannot be edited. It is possible, although not recommended, to turn off the Run Log by deselecting its checkbox in the Edit User Preferences window, available from the **Options** menu.



The Run Log screen is a dialog box titled "Event Log for <<run name>>". It contains the following fields and controls:

- Close button
- Description: [Text Field]
- Author: [Text Field]
- Date: <<Time and Date>>
- User Name: <<name>>
- Project Name: <<name>>
- Method Name: <<name>>
- Run Name: <<name>>
- Run Operator: <<name>>
- <<run description>>

Figure 7-10. Run Log Screen

7.5 POST RUN Screen

The Post Run screen enables peak annotation (“tagging”) and data export. To enter the Post Run screen, open a run file in the Browser screen, or when a run is finished choose **PostRun** from the toolbar. Two chromatograms are viewed: the main chromatogram in the lower large window and the reference chromatogram in the upper right window. In the upper reference chromatogram, use the sliding sidebar to make scaling adjustment.

Note: The Values at Cursor window in the upper left corner will list fewer traces if you are not using the QuadTec multiwavelength detector.

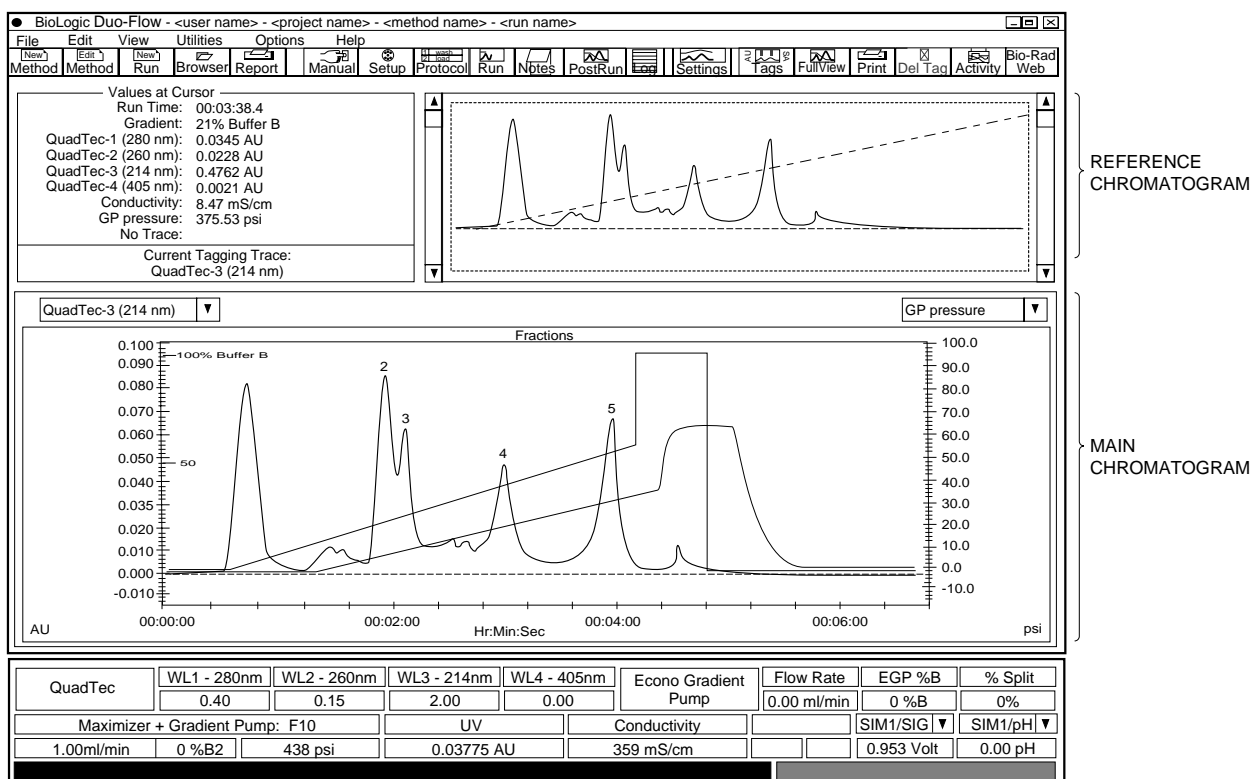


Figure 7-11. Post Run Screen

7.5.1 Resizing

The main chromatogram shows the current “Zoom” region. To resize the image (or “zoom in” to a particular region of the chromatogram), either change the axes scale in the reference chromatogram, or use the “rubber band” controls on either chromatogram. To use the rubber band controls, click and hold the left mouse button. Drag it across the chromatogram region. Release the mouse button and the main chromatogram will be resized. Click on the **Full View** button in the toolbar to return to normal view.

7.5.2 Chromatogram Information (Values at Cursor)

The top left corner of the PostRun screen contains information about the chromatogram. The position of the vertical bar within the main chromatogram dictates the displayed information. Run time, the absorbance (AU) and conductivity (mS/cm) values, gradient progression (%B), and the current tagging trace are displayed for the peak selected by panning the vertical bar across the chromatogram. To view the vertical bar, move the cursor inside the main chromatogram.

7.5.3 Annotating (“tagging”) the Chromatogram

Tags can be placed on any trace. To assign tags to a trace, and to view all assigned tags and their data, use the Post Run Tags window, shown below. To display the Post Run Tags window, click once on the **Tags** button in the toolbar or from the Edit drop down menu, select **Tag and Trace Options**.

In the Post Run Tags window, select a trace by clicking on an Active Trace radio button. Trace visibility and tag visibility can also be selected from this box. Tag styles are specified in the upper right box. Peaks can be annotated with a sequential tag number (1,2...), a user-defined name (user tag name), with the trace value, or the run time.

Trace	Trace Visibility	Tag Visibility	Active Trace
(%) Buffer B	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="radio"/>
UV (AU)	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="radio"/>
Conductivity (mS/cm)	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="radio"/>
GP pressure (psi)	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="radio"/>
(no trace)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="radio"/>
(no trace)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="radio"/>
(no trace)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="radio"/>
(no trace)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="radio"/>

Tag Style for active trace

☒ Sequential Tag Number

☐ User Tag Name

☐ Trace Value (Y axis value)

☐ Run Time (X axis value)

Ok

Cancel

Tag #	Tag name	Retention time (h:m:s)	Volume (ml)	Buffer B (%B)	UV (AU)	Cond. (mS/cm)	GP pres. (psi)	(no trace)	(no trace)	(no trace)	(no trace)	(no trace)	Tube #
1	Myoglobin	00:00:32	2.2	0	0.07992	1.496	N/A	N/A	N/A	N/A	N/A	N/A	0.07992
2	Conalbumin, peak 1	00:01:41	6.7	18	0.08617	7.999	N/A	N/A	N/A	N/A	N/A	N/A	0.08617
3	Conalbumin, peak 2	00:01:48	7.2	20	0.05921	9.025	N/A	N/A	N/A	N/A	N/A	N/A	0.05921
4	Ovalbumin	00:02:44	11.0	34	0.03799	16.472	N/A	N/A	N/A	N/A	N/A	N/A	0.03799
5	Soybean Trypsin Inhibitor	00:03:30	14.0	46	0.06844	22.308	N/A	N/A	N/A	N/A	N/A	N/A	22.308

Figure 7-12. Post Run Tags for UV Detector
(Note: Additional Traces will be shown when the QuadTec Detector is being used.)

To change a tag name in the Post Run screen chromatogram, move the cursor over the tag and note that the cursor changes. Double click on the tag and in the window that appears, enter the tag name. To deselect a tag, highlight the undesired tag and press DelTag from the button bar. To remove all tags, select “Delete all tags” from the **Edit** drop-down menu.

7.5.4 Entering Activity Data

The Activity Trace Editor permits data collected by a separate offline method to be included with data collected by the DuoFlow method. For example, if fractions collected by the DuoFlow are also analyzed by an ELISA method or for radioactivity, CPM, or DPM, the data collected by these assays can be entered into the Activity Trace Editor and a trace will appear on screen reflecting the activity for each fraction.

From the Browser screen, select the run for which you wish to enter the post run activity data. From the Post Run screen that is displayed:

1. Select the **Activity** icon from the toolbar. A box will appear listing each fraction collected in the opened run. (See Figure 7-13.)
2. Enter activity values for each fraction and enter the desired units.
Check the Show Activity Trace box to display the data in the Post Run screen.
3. The activity values entered will appear in the chromatogram information screen and as a chromatogram trace. (See Figure 7-14.)

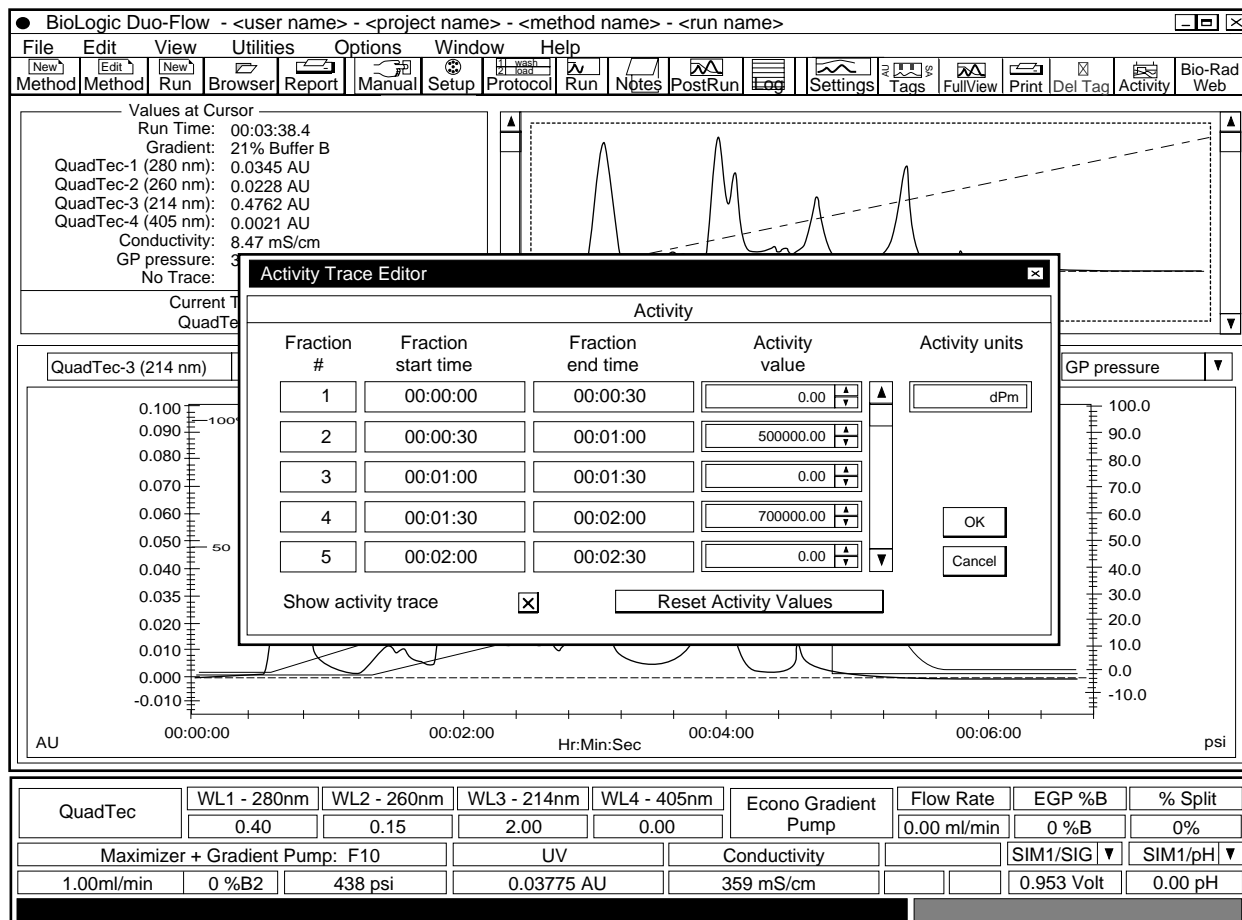


Figure 7-13. Activity Trace Editor

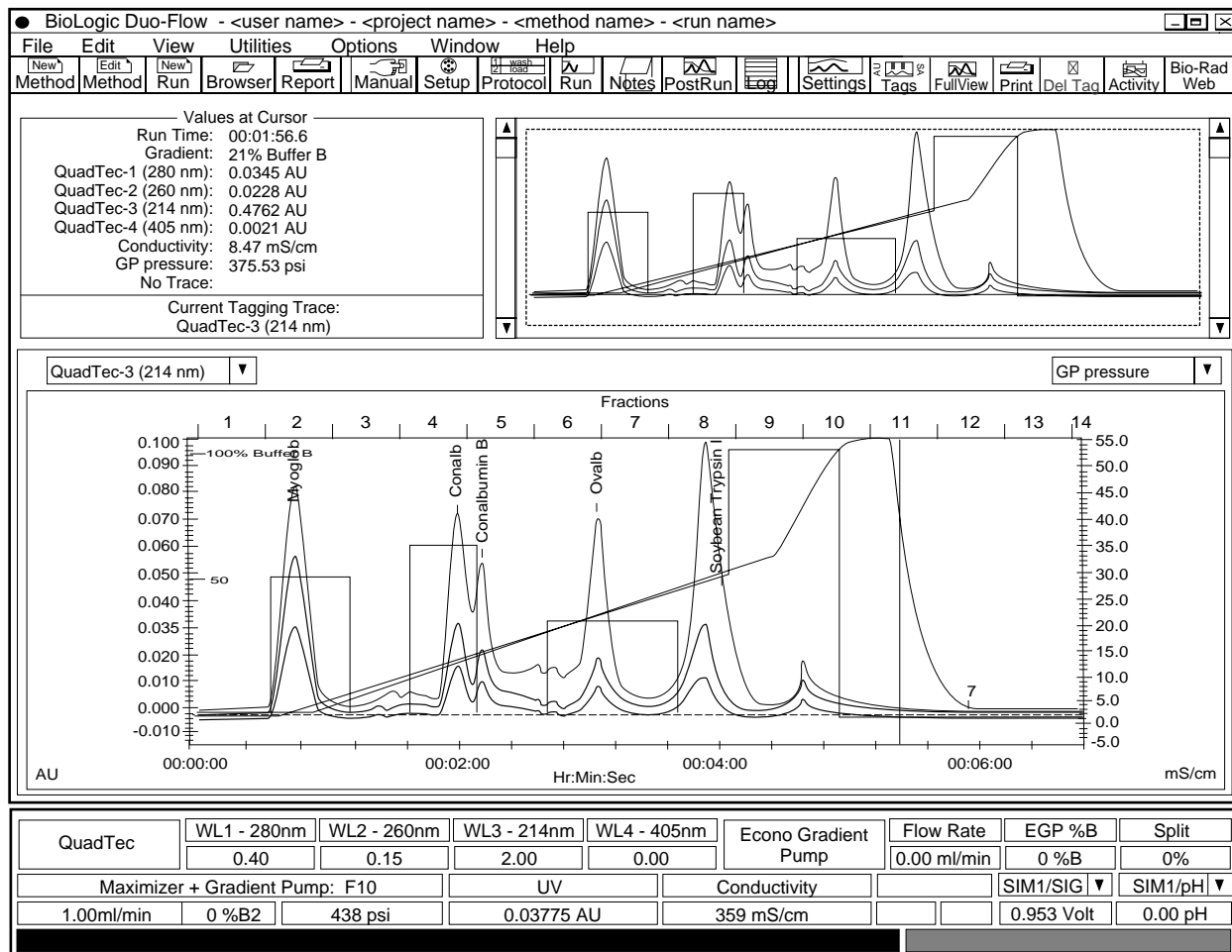
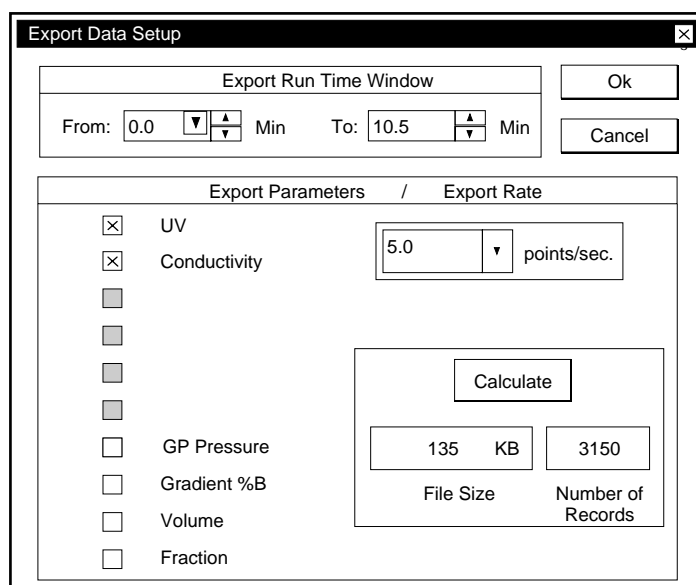


Figure 7-14. Activity Trace

7.5.5 Exporting Chromatogram Data to other Software Applications

To transfer data, go to the Post Run screen of the desired run. Select **Export Data** from the File drop-down menu (See Figure 7-15, Export Data Setup screen). From this screen, choose the run time and the data trace(s) that will be exported. Although data is collected at a rate of five points per second, it can be exported at a user-defined rate. Data is exported in an ASCII.TXT file format. The file name is user-defined and can be saved anywhere on the hard drive. Note that the default file is called "Export" and is located in the directory "Biologic/data/export". When importing the data into Excel, choose comma delimited.



The "Export Data Setup" dialog box is divided into two main sections. The top section, titled "Export Run Time Window", contains "From:" and "To:" fields with spinners, both set to 0.0 and 10.5 respectively, followed by "Min". To the right are "Ok" and "Cancel" buttons. The bottom section, titled "Export Parameters / Export Rate", contains a list of parameters with checkboxes: UV (checked), Conductivity (checked), and four unlabeled checkboxes (all unchecked). To the right of this list is a spinner set to 5.0 with the label "points/sec.". Below the list is a "Calculate" button. At the bottom right, there are two boxes: "135 KB" labeled "File Size" and "3150" labeled "Number of Records".

Parameter	Selected
UV	Yes
Conductivity	Yes
	No
	No
	No
	No
GP Pressure	No
Gradient %B	No
Volume	No
Fraction	No

Export Rate: 5.0 points/sec.

Calculate

File Size: 135 KB Number of Records: 3150

Figure 7-15. Export Data Setup Screen

7.5.6 Exporting Chromatogram Images

Chromatographic images can be exported into other applications, such as word processing programs. Before transferring an image, modify the chromatogram by selecting the desired traces and set the scale and zoom region in the PostRun screen. This should be done now since changes cannot be made to the file once it is transferred out of the DuoFlow program. The image in the main chromatogram will be the exported image.

Select **Export Chromatogram Image** from the File drop-down menu (see Figure 7-16). The image is exported as a Windows Meta File (*.wmf) format. In general, the Enhanced Meta File format is used for transfer into a Windows-based application.

Alternatively, a chromatogram can be copied to the Windows clipboard and pasted into another Windows program. Choose **Copy zoom Chromatogram to clipboard** from the Edit drop-down menu.

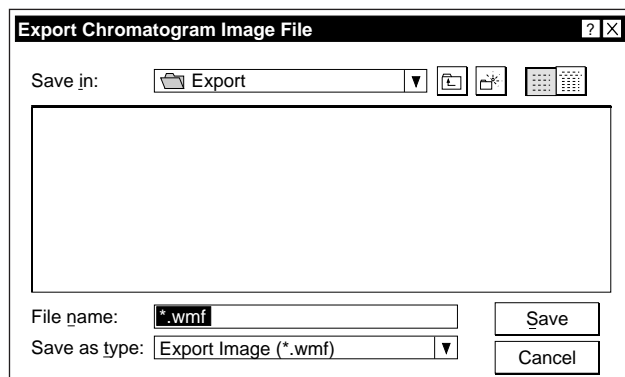


Figure 7-16. Exporting a Chromatographic Image

8.0 SAMPLE LOADING

The BioLogic DuoFlow system supports several methods for sample loading. Typically a sample is loaded through a static fixed volume loop, as shown in Chapter 4. When large volume sample injections are required, the following options are available:

- sample loading through the Workstation pump with an SV5-4 valve
- sample loading using the DynaLoop sliding-piston loop
- sample loading onto a low pressure column using the Econo Gradient Pump (EGP) or EP-1 Econo pump as an auxiliary (Aux) pump
- multiple sample loading using either an AVR9-8 valve or DynaLoop

8.1 SAMPLE LOADING THROUGH THE WORKSTATION PUMP WITH AN SV5-4 VALVE

Samples may be loaded directly through the Workstation pump, as long as the sample is first filtered through a 0.45 μm filter. Use a sanitizing solution to remove protein residues after the sample has been loaded; protein residues might otherwise reduce piston seal lifetime. Consult the DuoFlow online help for additional information.

8.1.1 System Setup

When loading sample through the Workstation pump, use an SVT3-2 or SV5-4 valve to facilitate sample loading and cleaning. Plumb the selected valve to inlet A on the pump. To prevent air from entering the system, unused ports on the valve should be closed with the 1/4-28 plugs supplied in the DuoFlow Fittings kit. In the Setup screen identify inlet A (left lower side of screen) as the sample inject valve and inlet B as the elution buffer.

In Figure 8-1, one port of the SV5-4 valve is dedicated to the sample, another to running/equilibration buffer, a third to storage solution (such as 20% ethanol (EtOH)), and the fourth to a sanitizing solution (such as 1M sodium hydroxide (NaOH)). The elution buffer is plumbed directly into pump B and is configured as inlet B in the Setup screen.

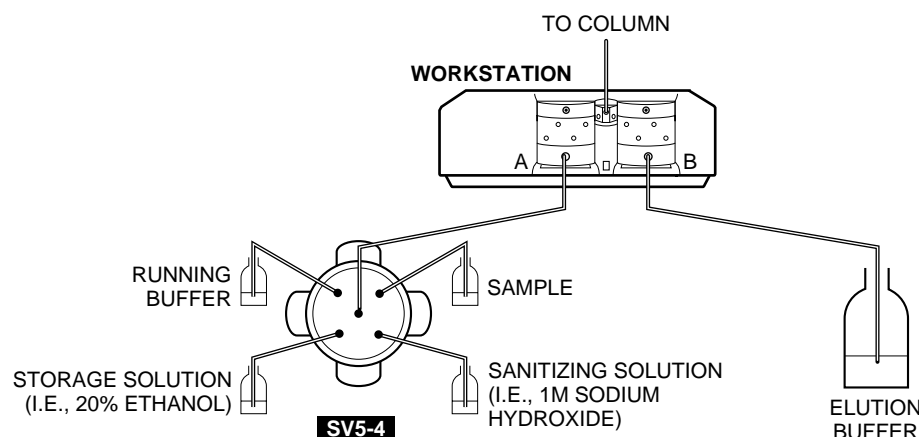


Figure 8-1. Sample Loading through the Workstation Pump

8.1.2 Writing the Protocol

The following steps should be programmed within the protocol, as shown in the figure below:

1. Select **Isocratic Flow** to establish the running conditions. Enter the desired flow rate and time required to equilibrate the system.
2. Select **Load/Inject Sample**, and specify Direct Inject and choose pump A as the inlet through which the sample will be delivered. Enter the volume of sample and the flow rate for the injection step. The step starting time or volume appears to the left of the options buttons.
3. Select **Isocratic Flow** and specify a running buffer. Ensure that all the sample is loaded onto the column by flushing with three to five volumes of inlet tubing length. There is approximately 20 μ l in 1 cm of 1/8" (3.2 mm) OD tubing. For example, for 10 cm of 1/8" (3.2 mm) OD tubing, program an isocratic flow step of 1.0 mL. (10 cm) (0.020 mL/cm) 5 = 1mL
4. Select **Change Valve** and define the step to move the AVR7-3 sample inject valve to the Purge position, taking the column off line. Select **Isocratic Flow** and run 10 ml of buffer B through pump B. This will ensure that the mixer has been removed of any protein residue. Select **Isocratic Flow** again to run 10 ml of running buffer through pump A to establish running buffer conditions.
5. Select **Change Valve** to move the AVR7-3 sample inject valve back to the Load position to begin the run.
6. Continue writing your desired separation protocol.
7. For sanitization, 1.0M sodium hydroxide (NaOH) may be used. Be sure to wash with water to remove the base. Refer to Chapter 11, Maintenance, for information on how to store the DuoFlow system.

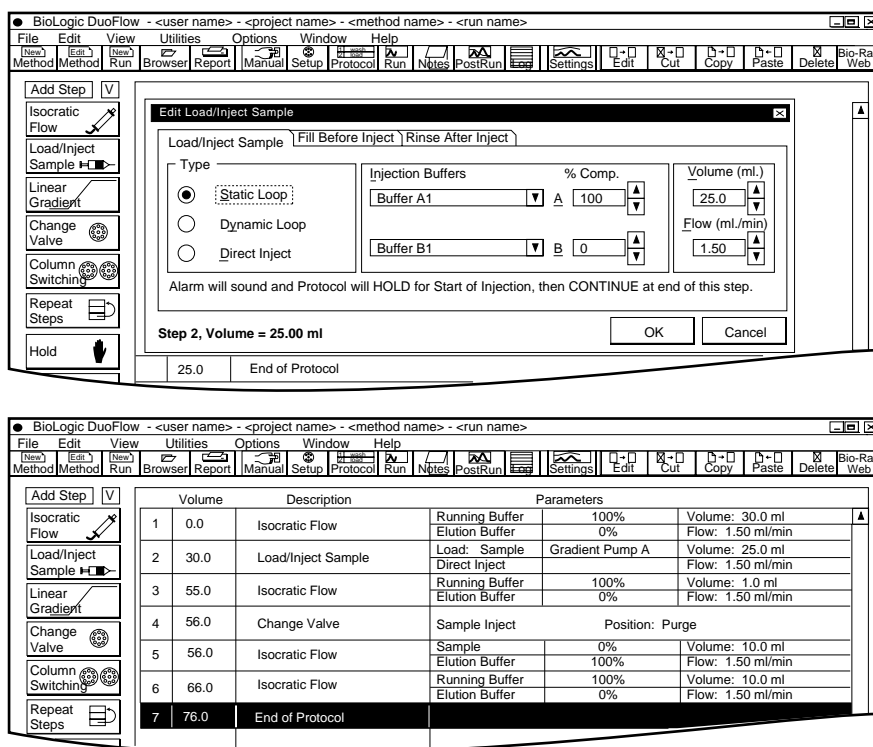


Figure 8-2. Writing a Protocol for Sample Injection through the Workstation pump

8.2 SAMPLE LOADING USING THE DYNALoop SLIDING-PISTON LOOP

The DynaLoop, which is available in 25 ml and 90 ml sizes, is used to load large volumes of sample directly onto chromatography columns. These loops have a sliding piston and function very much like a syringe. The sample is inserted into the DynaLoop's sample end connector. The loading of the sample pushes the DynaLoop's sliding seal assembly towards the buffer end connector. **Note:** While other dynamic loops may be used, the application discussed in this section applies specifically to the DynaLoop.

8.2.1 System Setup

When plumbed directly to the AVR7-3 inject valve, the DynaLoop functions just like a static sample loop. The sample end of the DynaLoop should be plumbed to port 3 and the buffer end to port 6 of the Inject valve. The valve's operation and sample loading are controlled automatically, simplifying the sample injection process and insuring precise sample loading and gradient formation.

The DynaLoop may be filled either manually with a syringe or automatically using an auxiliary pump such as an Econo Gradient Pump (EGP) which is controlled by the DuoFlow system or the EP-1 Econo pump.

- When using a syringe to fill a DynaLoop, the syringe should be connected to port 2 of the AVR7-3 valve. However, using a 1/4x28 to female Luer adapter between the syringe and the valve makes filling the loop much easier.
- When using the auxiliary pump to fill the loop, the pump's outlet plumbing should be connected directly to port 2 of the inject valve.

Consult the DynaLoop Instruction Manual or DuoFlow online help for additional information. The figure below shows how to set up a DynaLoop.

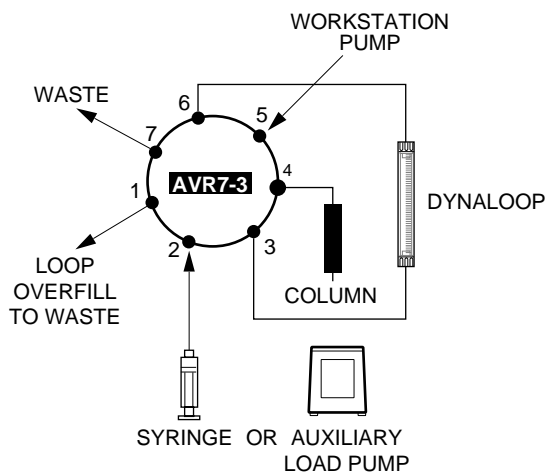


Figure 8-3. Plumbing the DynaLoop for use with an Inject Valve

8.2.2 Writing the Protocol

Before running a protocol which involves the use of a DynaLoop, be sure to purge air from the lines, as discussed in the DynaLoop Instruction Manual, Chapter 2, Installation. Then follow the guidelines which apply to your application.

The following steps should be programmed within the method protocol to manually load a sample:

1. Ensure that the column is properly equilibrated by using an **Isocratic Flow** step.
2. From the Manual screen, set the AVR7-3 sample inject valve to its Inject (I) position and allow the sliding seal to contact the sample end fitting on the DynaLoop. Then set the valve to the Load (L) position.
3. Load sample into the DynaLoop by using a syringe. As sample is loaded into the loop, it displaces the buffer that was used to purge the unit. This design means the DynaLoop cannot be overfilled. When sufficient volume of sample for a run or series of runs is loaded into the DynaLoop, stop the filling process. For a series of partial volume injections after one loading sequence, fill the DynaLoop with about 2 percent extra sample. Leave the syringe in the Inject port to minimize the introduction of air into the DynaLoop.
The sample is now ready for injection, and the protocol can be programmed.
4. In the Protocol screen, select **Load/Inject Sample**. In the Load/Inject Sample window, note that the AVR7-3 valve will automatically move to the "Inject" position at the start of the step and to the "Load" position at the end of the step.
 - a. In the Load/Inject Sample window, specify Dynamic Loop.
 - b. In the "Injection Buffer" area of the window, select the buffer(s) and percent (%) composition to be used to inject the sample onto the column.
 - c. In the "Volume (ml)" field, enter the sample volume.
 - d. In the "Flow (ml/min)," enter the flow rate of the Workstation pump that will be used to inject the sample onto the column.
5. Continue writing the separation protocol.

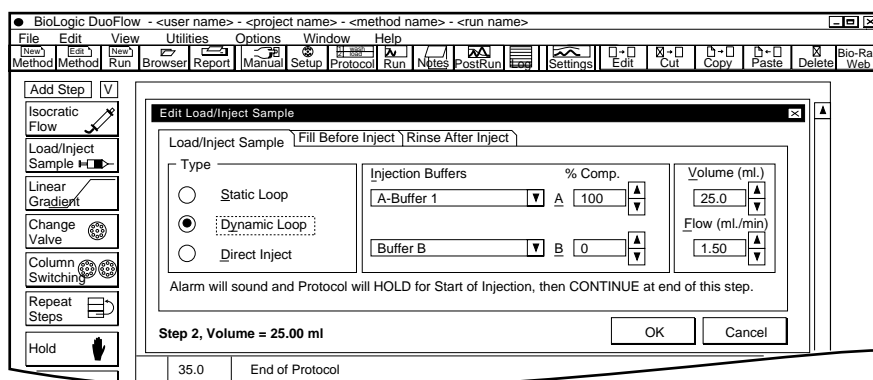


Figure 8-4. Load/Inject Sample Window for a Manual Load/Inject of the Sample

The following steps should be programmed within the method protocol to automatically inject a sample using an auxiliary pump:

1. Connect the Aux pump. The EGP pump connects to the Instrument bus. If you are using the EP-1 Econo pump, consult Section 8.3.1.
2. In the Setup screen, select Aux Load Pump and AVR7-3 valve in addition to the other device and instruments you have connected to the system.
3. In the Protocol screen, program an **Isocratic Flow** step that is long enough for the auxiliary pump to load the desired quantity of sample into the DynaLoop. For example, if you are loading 25 ml of sample at an auxiliary pump flow rate of 5 ml/min, you will need at least a 5 minute step prior to the sample loading step. Otherwise the protocol will fail the automatic validation done prior to the run. The flow rate of the DuoFlow pump is not critical, so you may set a low flow rate (e.g., 0.1 ml/min) to minimize buffer waste. It is important that either the time or volume length of this first step is of sufficient duration to allow the auxiliary pump to fill the DynaLoop.
4. In the Protocol screen, select **Load/Inject Sample** to program the DuoFlow system to automatically fill and inject the DynaLoop sample. From the Load/Inject Sample window,
 - a. Select the Dynamic Loop as the type of loop to be used.
 - b. Select **Fill Before Inject**. This instructs the auxiliary pump to load the sample into the DynaLoop. Note that the auxiliary pump flow rate is not under DuoFlow control. The flow rate is used by the system when validating the protocol before the run. Because the DuoFlow system only starts/stops the auxiliary pump, the correct flow rate must be set at the auxiliary pump. The flow rate of the Econo pump is set from the pump and is recorded in the yellow data entry boxes of the Fill Sample section of the Load/Inject Sample dialog box. Note that the rinse function is not available when the DynaLoop is being used.
 - c. In the Fill Sample Loop area of the window, select the sample to be loaded and enter its volume and flow rate of the auxiliary pump.
 - d. In the Inject Sample area of the window, select the Injection Buffers, the buffer composition, the flow rate of the Workstation pumps, and the sample volume to be injected onto the column.The DuoFlow system will now automatically control the loading and injection of the sample. Note that the rinse function is not available when the DynaLoop is being used.
5. Continue writing your desired separation protocol.

During the run, the valve functions as follows:

- **Load.** While in this position the valve connects ports 5 and 4 for equilibration of the column and for sample elution. In this position, sample loop is loaded to the desired volume via port 2 and buffer is expelled from the dynamic loop through port 1.
- **Inject.** While in this position the valve connects ports 5 and 6 and ports 3 and 4 for applying the sample onto the column. The flow from the Workstation pump forces the sliding piston to expel the sample onto the column.
- **Purge.** While in this position the valve connects ports 5 and 7 and allows purging or buffer changes of the Workstation pump without the need to remove the column from the system.

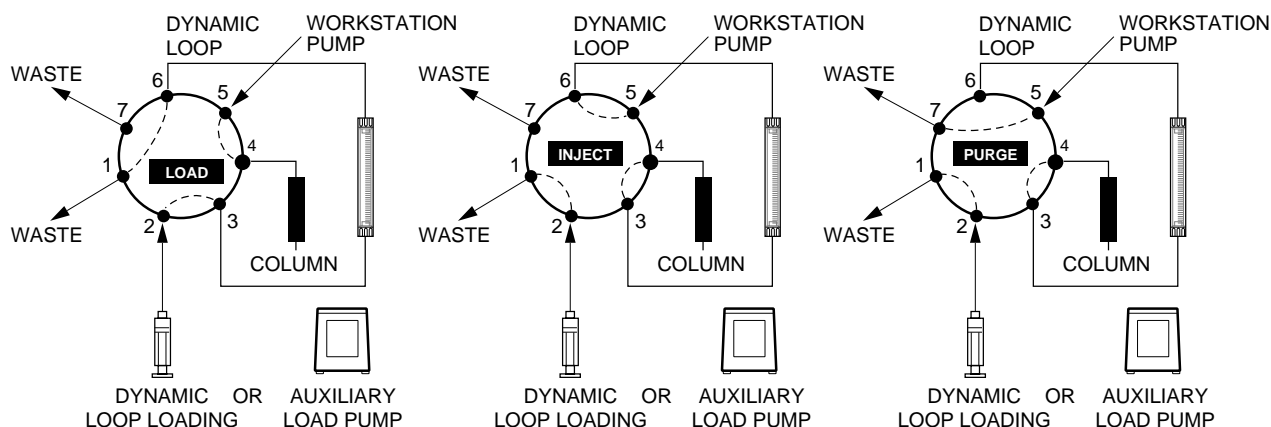


Figure 8-5. Valve Positions During a Run using the DynaLoop

8.3 SAMPLE LOADING ONTO A LOW PRESSURE COLUMN USING AN AUX PUMP

The Econo Gradient Pump (EGP) and the EP-1 Econo pump are ideal for loading samples onto a low pressure column. When using a low pressure column, such as an Econo-Pac cartridge, remove the 40 psi backpressure regulator from the post-column position. Place it between the Workstation pump and the mixer. This will allow the check valves to properly seat and ensure pump flow performance. In addition, low pressure columns may burst if the backpressure regulator is placed after the column.

8.3.1 System Setup

The DuoFlow can be used with the following auxiliary pumps:

- Econo Gradient Pump (EGP). This pump connects directly to the system Instrument bus.
- Model EP-1 Econo Pump.

The EP-1 Econo pump must contain firmware version 2.12 or higher to function with the DuoFlow system. To confirm the firmware version, simultaneously press and hold down the Direction key and the down arrow key on the front panel of the EP-1 Econo pump. If the EP-1 Econo pump does not have the correct firmware version, contact Bio-Rad for information on how to upgrade the pump.

The Econo pump receives start/stop signals only. The flow rate of the EP-1 Econo pump is set from the pump, and the flow rate is recorded in the yellow data entry boxes of the Load/Inject Sample dialog box.

Note: The DuoFlow system does not control the flow of the EP-1 Econo pump, but sends only starts/stops to the Aux pump. The correct flow rate must be set at the Aux pump.

- Other external pumps.

The DuoFlow system sends a TTL signal to control an external pump. This signal is normally TTL high (5 volts). The DuoFlow system commands the external pump to run by holding Pin 4 "low". Any pump accepting this signal may be used with the DuoFlow system as a sample loading pump. Refer to your pump's separate documentation when connecting the pump to the DuoFlow.

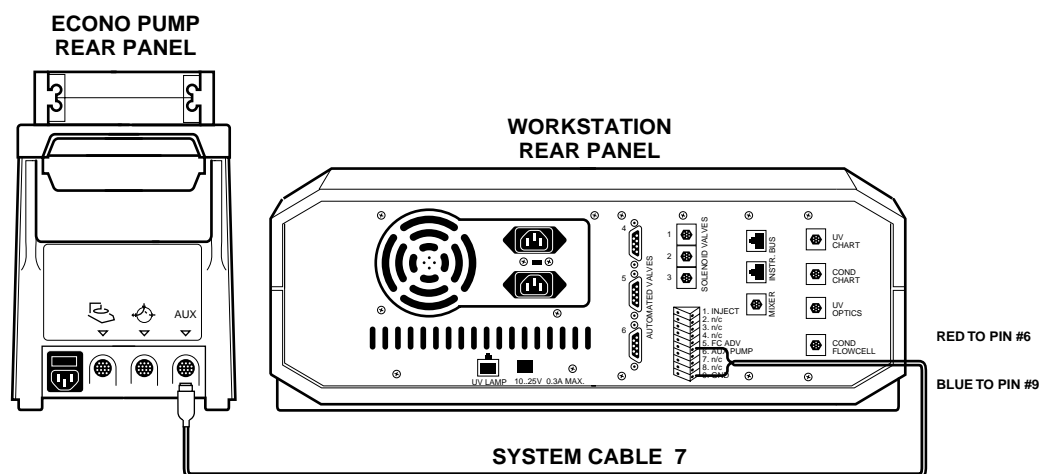


Figure 8-6. Connecting an EP-1 Econo Pump to the DuoFlow Workstation

To connect the EP-1 Econo pump with the DuoFlow, make the following cable connections:

1. Obtain **System Cable 7**.
2. Connect the red and blue wires on the cable to the DuoFlow Workstation. (See Figure 8-6.)
Note: Cut short all other wires and isolate with tape.
 - a. Connect the red wire to pin 6 of the Aux connector.
 - b. Connect the blue wire to pin 9 of the Aux connector.
3. Plug the 8-pin mini-DIN connector into the Aux connector on the rear panel of the EP-1 Econo pump.

PharMed tubing is recommended for use in the pumpheads of the Econo Gradient Pump or the EP-1 Econo pump. For additional information on these pumps, consult their instruction manuals. The AVR7-3 inject valve is plumbed without a sample loop. Plumb the AVR7-3 valve as shown below, with the Aux pump connected to port 3 of the inject valve.

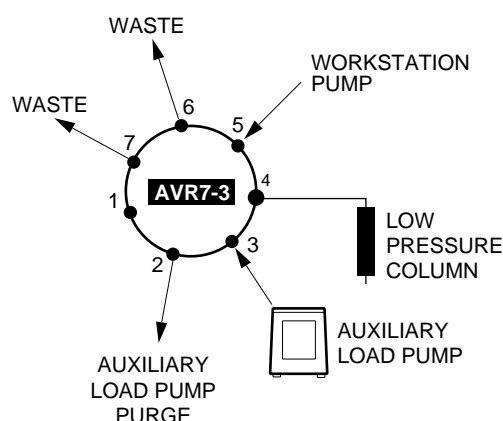


Figure 8-7. Plumbing an AVR7-3 Inject Valve with an Auxiliary Load Pump

In the Setup screen, select the AVR7-3 valve as the Sample Inject Valve and add the Aux pump to the Setup list. When using an auxiliary pump to load sample directly to an AVR7-3 sample inject valve, it is possible to have a valve, such as an SVT3-2 valve, assigned as the “Aux Pump Inlet” to allow switching between samples and rinse solutions.

8.3.2 Writing the Protocol

The following steps should be programmed in the protocol, as shown in Figure 8-8.

1. Ensure that the column is properly equilibrated by using an Isocratic Flow step.
2. When you select **Load/Inject Sample**, specify Direct Inject and choose Aux pump as the inlet through which the sample will be delivered. Note the message displayed in yellow, “Aux Pump Flow Rate not under Biologic control,” and that the flow rate also is displayed in yellow. This is to serve as a reminder that the DuoFlow only starts/stops the Aux pump and that the actual flow rate must be set at the Aux pump.
2. Enter the volume of sample and the flow rate for the injection step. The step starting time or volume appears to the left of the options buttons.

Note: The DuoFlow system does not control the flow of the EP-1 Econo pump. The correct flow rate must be entered at the EP-1 Econo pump faceplate.

- Continue writing your desired separation protocol.

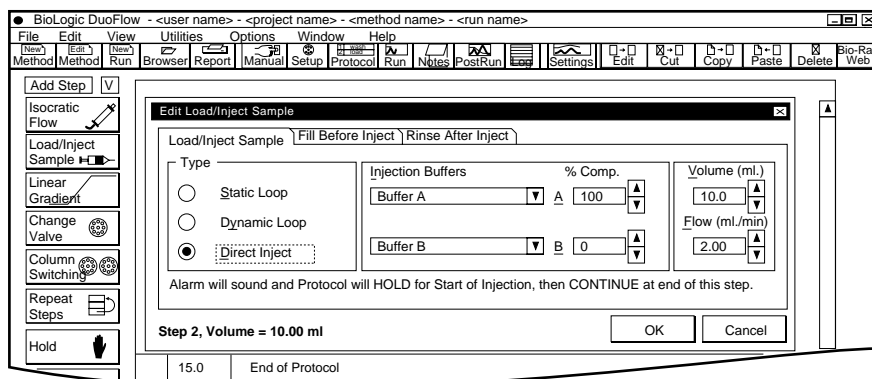


Figure 8-8. Writing the Protocol: Direct Sample Loading

In the configuration shown below, the valve operates as follows:

- Load. While in this position the valve connects ports 5 and 4 for equilibration of the column and for sample elution.
- Inject. While in this position the valve connects ports 3 and 4 to load sample onto the column.
- Purge. While in this position the valve connects ports 5 and 7 and allows purging or buffer changes without the need to remove the column from the system.

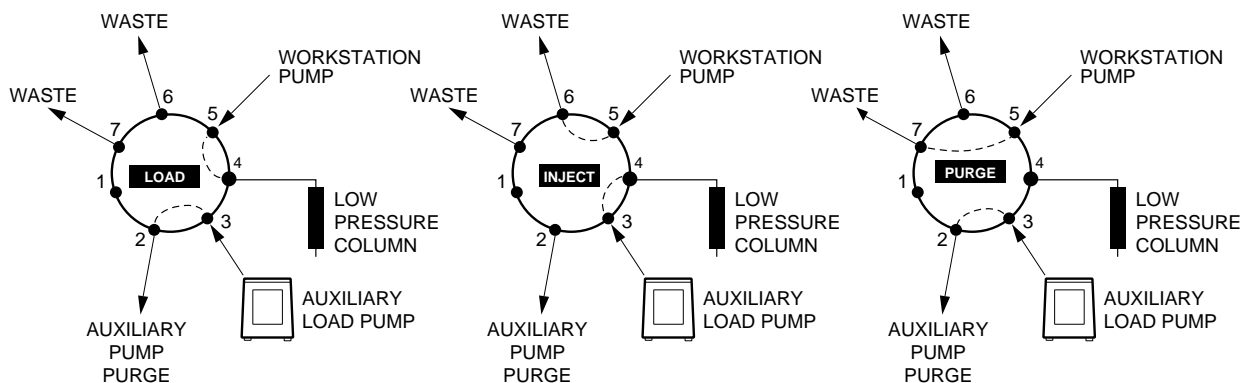


Figure 8-9. AVR7-3 Valve Positions During a Run with Direct Sample Loading and Injection

8.4 MULTIPLE SAMPLE LOADING

With the addition of the Econo Gradient Pump or an EP-1 Econo pump as an Aux pump and an AVR9-8 as an Aux pump inlet valve, the system can load up to 7 samples sequentially.

8.4.1 System Setup

Connect the Aux pump to the system (connect the EGP to the Instrument bus; connect the EP-1 Econo Pump as described in Section 8.3.1.) In the Setup screen, alias the AVR9-8 as an Aux Pump Inlet. Name seven positions to identify each sample, and name one position "Rinse." The rinse solution will clean the sample loop between injections. Add the Aux pump to the Setup list.

Note: Some sample will be lost in the process of pulling sample from a remote beaker. Be sure to factor in the volume that is required to fill all buffer lines leading up to the top of the column when programming the injection step.

When using a low pressure column such as an Econo-Pac cartridge, remove the backpressure regulator from a post-column position. Place it between the pump and the mixer. This will allow the check valves to properly seat. In addition, low pressure columns may burst if the backpressure regulator is placed after the column.

See the illustration below to assist in setting up the system. Be sure to keep tubing lengths to a minimum when plumbing the system. This will minimize sample loss.

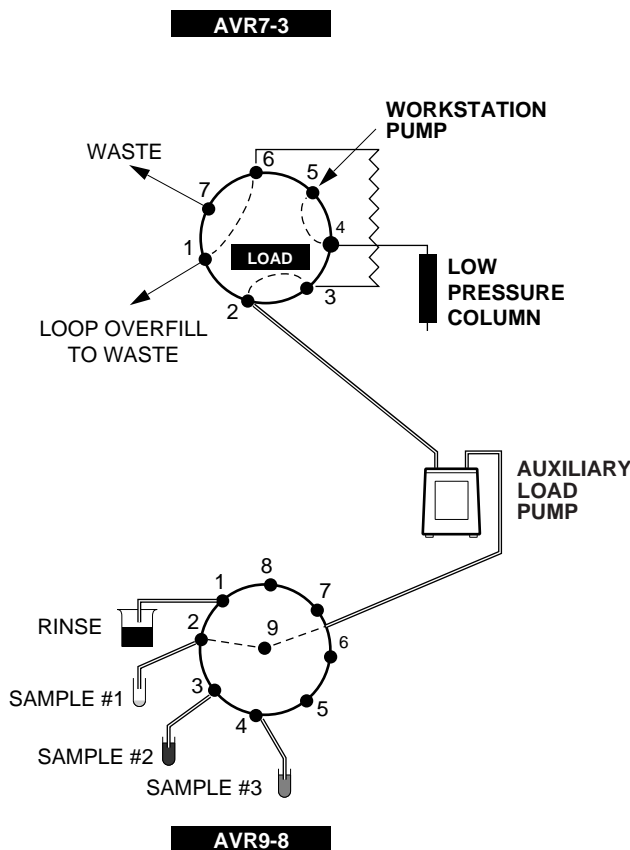


Figure 8-10. Using an Auxiliary Load Pump and an AVR9-8 Valve to Load Multiple Samples

8.4.2 Writing the Protocol

The following steps should be programmed within the method protocol:

1. Ensure that the column is properly equilibrated by using an Isocratic Flow step.
2. The flow rate of the Econo Gradient Pump is controlled by DuoFlow software. The DuoFlow system only starts/stops the Aux pump; set the correct flow rate at the Aux pump front panel. Set the correct flow rate on the EP-1 Econo pump and enter the flow rate in the yellow boxes of the Load/Inject Sample dialog box.
3. At the **Load/Inject Sample** step, specify Static Loop and choose “Fill before Inject” and “Rinse after Inject.” This automates the process of filling the sample loop using the Aux pump, injecting the sample using the Workstation pump, and then rinsing the sample loop using the Aux pump. Note the message displayed in yellow, “Aux Pump Flow Rate not under DuoFlow control,” and that the Flow rates also are displayed in yellow. This is to serve as a reminder that the actual flow rate must be set at the Aux pump, and the flow rate must be manually entered on the DuoFlow screen. Refer to the figure below.
4. Continue writing your desired separation protocol.

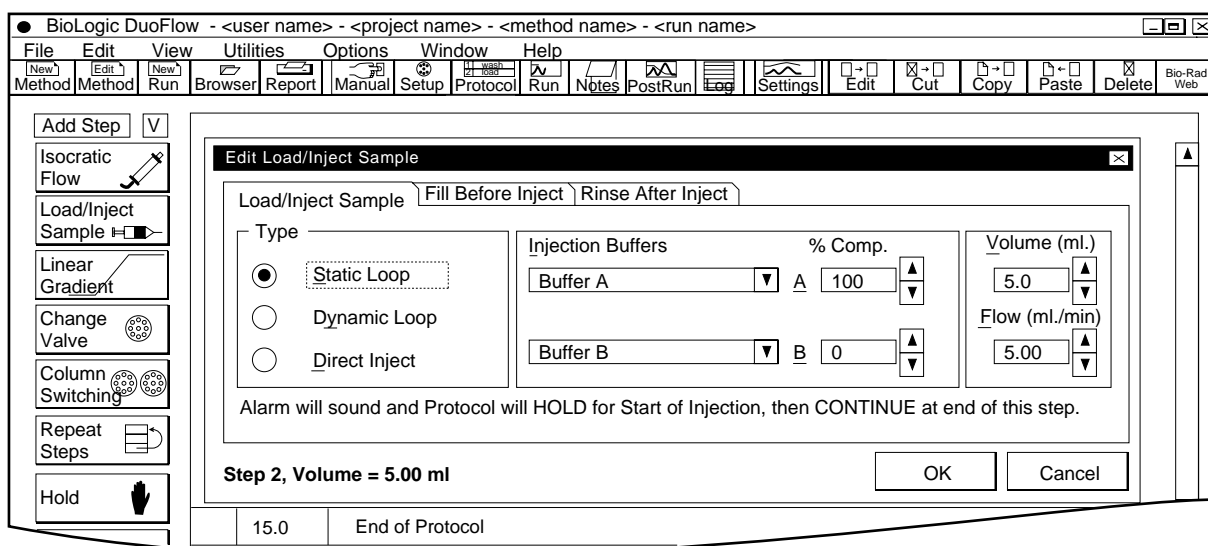


Figure 8-11. Load/Inject Sample Window for Loading Multiple Samples

8.5 MULTIPLE INJECTIONS OF A SINGLE SAMPLE

The DuoFlow system can be used in conjunction with the DynaLoop to run repeated injections of the same sample.

8.5.1 System Setup

Install the DynaLoop as described in Section 8.2.

8.5.2 Writing the Protocol

The following should be programmed within the protocol:

1. Ensure that the column is properly equilibrated by using an Isocratic Flow step.
2. In the **Load/Inject Sample** step, choose Dynamic Loop and enter the volume of sample to be injected during each run. At the initiation of the run, select Multiple Runs and the number of times you want to repeat the separation.

Note: The DuoFlow system does not sense how much sample is available in the sample loop, and will not indicate a programming error if you program more runs than you have sample for.

2. Continue writing your desired separation protocol.

9.0 MULTIPLE COLUMNS

The DuoFlow systems support the use of multiple columns. Refer to Chapter 2, Section 2.10, Columns and Column Fittings, for a description of columns and fittings offered by Bio-Rad. Several examples are discussed below.

9.1 COLUMN SWITCHING

For this application two AVR7-3 valves will be used: one as a sample injector, and the second used down stream as a column switcher. Alternatively, an SV5-4 valve may be used for sample loading (refer to Section 8.1) and one AVR7-3 valve for column switching.

9.1.1 System Setup

Figure 9-1 below shows the two valve positions when the injection valve is used for column switching. In this configuration, the sample from an upstream injection valve enters via port 5 and is diverted to either column using the load and inject positions of the valve. Although the purge position is not described in this application, it may be used in the conventional manner.

On the column switching valve, column 1 has its inlet connected to port 4 and its outlet to port 3. Column 2 has its inlet connected to port 6 and its outlet to port 1. Port 5 functions as a common inlet port, and port 2 functions as a common outlet port.

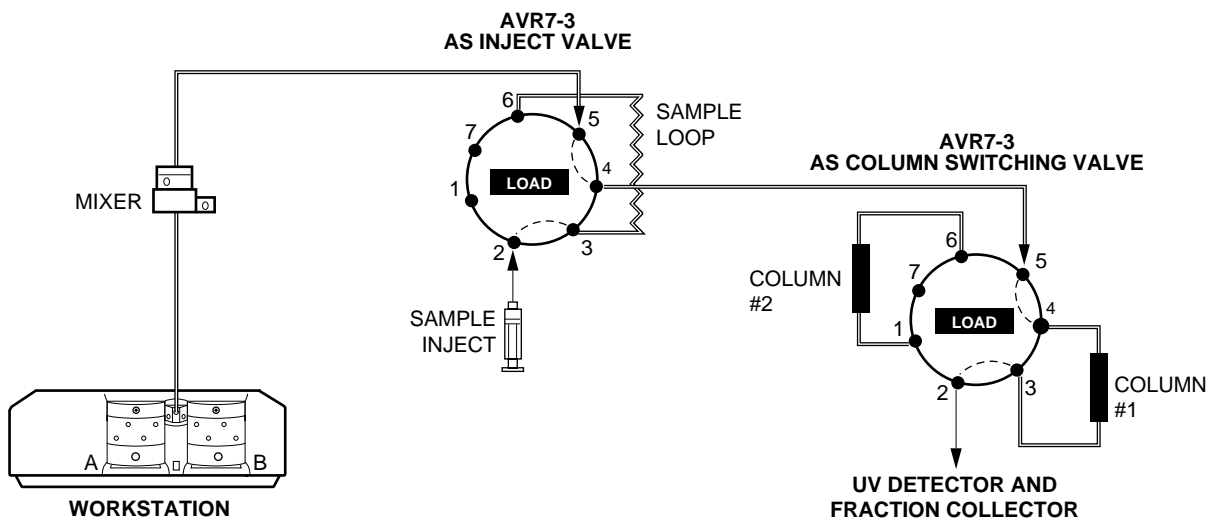


Figure 9-1. Column Switching using Two AVR7-3 Valves

In the Setup screen, use the default name of "Sample Inject" for the first AVR7-3 valve. Name the second AVR7-3 "column switcher." Enter the column names into positions 1 and 2, and name position 3 as "Purge."

Note that when solution is directed to column 1, this is similar to the "Load" position. When solution is directed to column 2, this is similar to the "Inject" position.

9.1.2 Writing the Protocol

The following steps should be programmed within the method protocol, as shown in the figure below.

1. Equilibrate both columns with the appropriate equilibration solution.
2. Select **Change Valve** to ensure that the column switching valve is in the appropriate position for loading the sample onto either column 1 or column 2.
3. Select **Load/Inject Sample** to load half the sample on to the appropriate column and continue to write the separation protocol for the sample and column.
4. Select **Change Valve** to ensure that the column switcher valve is in the appropriate position for loading sample onto either column 1 or column 2.
5. Use precautionary washes/rinses to prevent cross-contamination by selecting the purge position on the column switcher valve. This is used to clean the tubing between the two AVR7-3 valves. Alternatively, performing an isocratic flow step before directing flow to the other column may also be used. This later step is depicted in the figure below.
6. Continue writing your desired separation protocol.

● BioLogic DuoFlow - <user name> - <project name> - <method name> - <run name>

File Edit View Utilities Options Window Help

Method Method Run Browser Report Manual Setup Protocol Run Notes PostRun Log Settings Edit Cut Copy Paste Delete Bio-Rad Web

Add Step Isocratic Flow Load/Inject Sample Linear Gradient Change Valve Column Switching Repeat Steps Hold Pause Alarm Zero Baseline EGP Fraction Collection	1	0.0	Isocratic Flow	Buffer A	100%	Volume: 5.0 ml
				Buffer B	0%	Flow: 1.00 ml/min
	2	5.0	Change Valve	Column Switcher	Position: Column #1	
	3	5.0	Load/Inject Sample	Load: Sample		Volume: 1.0 ml
				Static Loop	Auto Inject Valve	Flow: 2.00 ml/min
	4	6.0	Linear Gradient	Buffer A	100% --> 30%	Volume: 6.0 ml
				Buffer B	0% --> 70%	Flow: 1.00 ml/min
	5	12.0	Isocratic Flow	Buffer A	0%	Volume: 10.0 ml
				Buffer B	100%	Flow: 2.00 ml/min
	6	22.0	Isocratic Flow	Buffer A	100%	Volume: 10.0 ml
				Buffer B	0%	Flow: 2.00 ml/min
	7	32.0	Change Valve	Column Switcher	Position: Column #2	
8	32.0	Isocratic Flow	Buffer A	100%	Volume: 10.0 ml	
			Buffer B	0%	Flow: 2.00 ml/min	
9	42.0	Load/Inject Sample	Load: Sample		Volume: 1.0 ml	
			Static Loop	Auto Inject Valve	Flow: 2.00 ml/min	
10	43.0	Linear Gradient	Buffer A	100% --> 30%	Volume: 6.0 ml	
			Buffer B	0% --> 70%	Flow: 1.00 ml/min	
11	49.0	Isocratic Flow	Buffer A	0%	Volume: 10.0 ml	
			Buffer B	100%	Flow: 2.00 ml/min	
12	59.0	Isocratic Flow	Buffer A	100%	Volume: 10.0 ml	
			Buffer B	0%	Flow: 2.00 ml/min	
	69.0	End of Protocol				

QuadTec	WL1 - 280nm	WL2 - 260nm	WL3 - 214nm	WL4 - 405nm	Econo Gradient Pump	Flow Rate	EGP %B	% Split
	0.40	0.15	2.00	0.00		0.00 ml/min	0 %B	0%
Maximizer Gradient Pump: F10			UV	Conductivity		SIM1/SIG	SIM1/pH	
1.00ml/min	0 %B	438 psi	1.003 AU	1.23 mS/cm		0.548 Volt	7.00 pH	

Figure 9-2. Protocol for using an AVR7-3 Inject Valve for Column Switching

9.2 RUNNING ONE SAMPLE SEQUENTIALLY OVER SEVERAL DIFFERENT COLUMNS

With the addition of two AVR9-8 valves, up to eight columns can be tested with a single sample.

9.2.1 System Setup

In the Setup screen, select "User Assigned Name" when naming each of the AVR9-8 valves. Name the first AVR9-8 valve "column inlet," and type in the names of the columns in positions 1-8. Name the second AVR9-8 valve "column outlet," and type in the names of the columns in positions 1-8.

The two column switching valves will be plumbed after the AVR7-3 inject valve. Position both valves to a convenient location on the rack to minimize tubing lengths. Connect port 4 of the inject valve to the common port of the "column inlet" valve. Connect the common port of the "column outlet" valve to the UV flow cell.

As with plumbing any column, be certain that all column inlet lines are free of air to avoid introducing air into the columns.

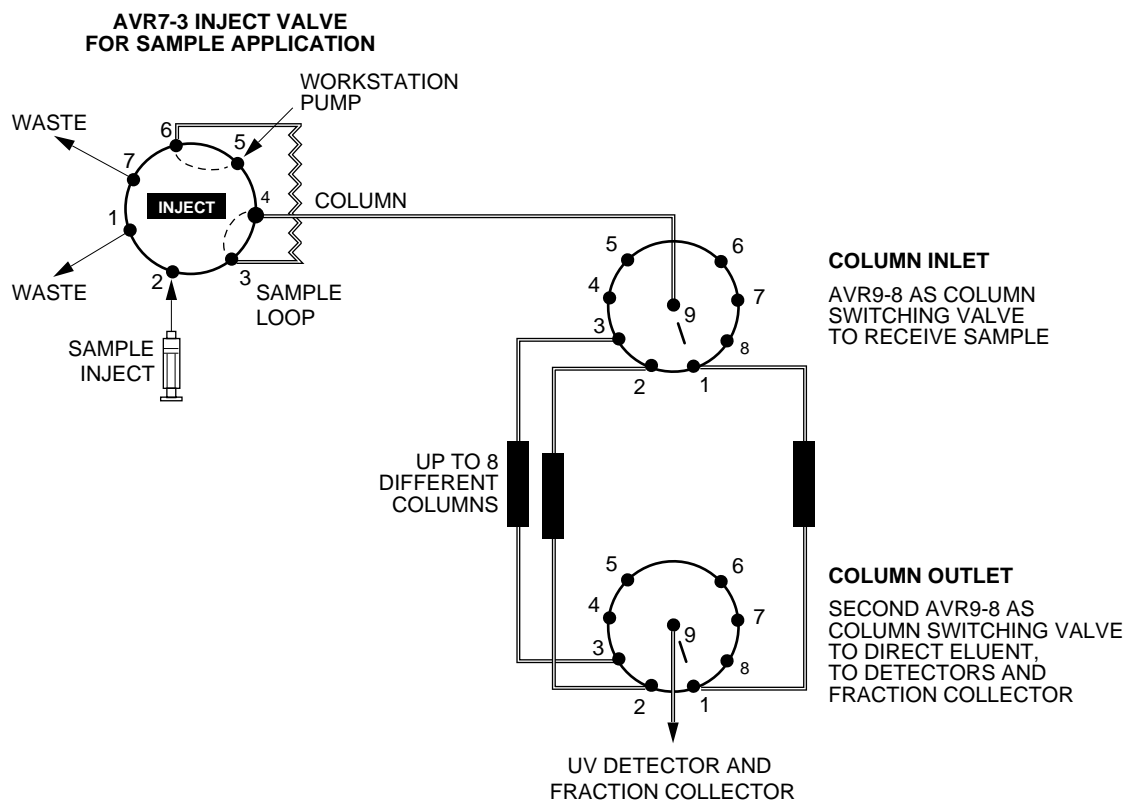


Figure 9-3. Column Switching using Two AVR9-8 Valves

9.2.2 Writing the Protocol

The following should be programmed within the protocol:

1. Begin the protocol by adding two Change Valve steps: one to position the column inlet at starting position, and the second to position the column outlet at the starting position. Be sure that these two positions are identical. For example if you wish to equilibrate column #1 with buffer A, the protocol should look as shown in the figure below.

Note: Lower the flow rate to less than or equal to 1.0 ml/min see steps 8 and 14 below) when performing sequential Change Valve steps.

2. Continue writing your desired separation protocol.

If multiple injections are performed in a single run, you may view or print each separation independently open up post run and highlight the section of the chromatogram you wish to view.

BioLogic DuoFlow - <user name> - <project name> - <method name> - <run name>						
File Edit View Utilities Options Window Help						
Method	Method	Run	Browser	Report	Manual	Setup
Protocol	Run	Notes	PostRun	Log	Settings	Edit
Cut	Copy	Paste	Delete	Bio-Rad Web		

Add Step	Time	Description	Parameters		
Isocratic Flow	1	0:00.0	Change Valve	column inlet	Position: Column 1
Load/Inject Sample	2	0:00.0	Change Valve	column outlet	Position: Column 1
Linear Gradient	3	0:00.0	Isocratic Flow	Buffer A 100%	Time: 10.0 min
Change Valve	4	10.0	Load/Inject Sample	Buffer B 0%	Flow: 2.00 ml/min
Column Switching	5	13.0	Linear Gradient	Load: Sample	Volume: 6.0 ml
Repeat Steps	6	19.0	Isocratic Flow	Static Loop	Flow: 2.00 ml/min
Hold	7	24.0	Isocratic Flow	Auto Inject Valve	Flow: 2.00 ml/min
Pause	8	29.0	Isocratic Flow	Buffer A 100% --> 30%	Time: 6.0 min
Alarm	9	29.1	Change Valve	Buffer B 0% --> 70%	Flow: 1.00 ml/min
Zero Baseline	10	29.1	Change Valve	column inlet	Position: Column 2
EGP	11	29.1	Load/Inject Sample	column outlet	Position: Column 2
Fraction Collection	12	32.1	Linear Gradient	Load: Sample	Volume: 6.0 ml
	13	38.1	Isocratic Flow	Static Loop	Flow: 2.00 ml/min
	14	48.1	Isocratic Flow	Buffer A 100% --> 30%	Time: 6.0 min
	15	48.2	Change Valve	Buffer B 0% --> 70%	Flow: 1.00 ml/min
	16	48.2	Change Valve	Buffer A 100%	Time: 10.0 min
	17	48.2	Isocratic Flow	Buffer B 0%	Flow: 2.00 ml/min
	58.2	End of Protocol			

Figure 9-4. Programming a Sequential Run

10.0 REVERSE FLOW CHROMATOGRAPHY

Using two AVR7-3 inject valves for reverse flow chromatography is advantageous for certain applications such as affinity chromatography, where minimal dilution during sample elution is important.

10.1 SYSTEM SETUP

In the Setup screen, select one AVR7-3 valve as the sample inject valve. Select a second AVR7-3 valve and name (alias) it as "Change Flow valve." On the "Change Flow valve," alias position 1 as Forward Flow, and alias position 2 as Reverse Flow. Leave Position 3 as is.

Connect the sample loop to ports 3 and 6 of the AVR7-3 inject valve. To the Change Flow valve, connect the column inlet to port 6, the column outlet to port 4. The Change Flow valve will require a connection between ports 1 and 2. Use approximately 8 cm of 1/16" (1.6 mm) OD tubing to make this connection. Keep this tubing length to a minimum to minimize sample dilution. Use minimal length of 1/16" (1.6 mm) OD tubing to connect port 4 of the AVR7-3 inject valve to port 5 of the Change Flow valve. Figure 10-1 shows how to set up this configuration.

If a low pressure column is used, remove the 40 psi backpressure regulator and place it between the pump and the AVR7-3 valve. This will allow the check valves to properly seat and ensure pump flow performance. In addition, low pressure columns will burst if the backpressure regulator is placed after the column.

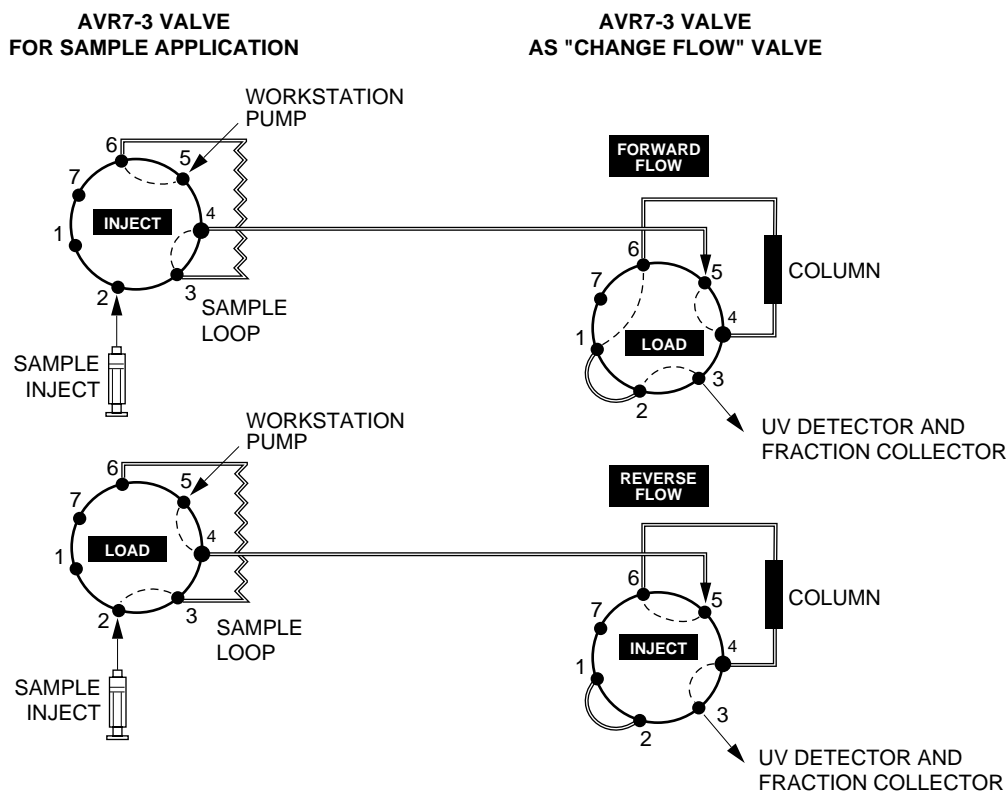


Figure 10-1. Injection Valve Plumbing for Use in Affinity Chromatography

10.2 WRITING THE PROTOCOL

The following steps should be programmed within the method protocol.

1. Ensure that the column is properly equilibrated by using an Isocratic Flow step.
2. Use a Change Valve step to place the Change Flow valve in the forward flow orientation.
3. Use the Load/Inject Sample step to load the sample loop and subsequently inject it onto the column. The AVR7-3 valve will automatically move to the inject position at the start of the step and return to the load position at the end of the step.
4. Use the appropriate buffer to wash all nonbinding components from the column.
5. When the sample is to be eluted, first use the Change Valve step to select the "Reverse Flow" valve position of the Change Flow valve. This directs the flow of buffer through the bottom of the column to elute sample components of interest in a concentrated form.
6. Continue writing your desired separation protocol.

BioLogic DuoFlow - <user name> - <project name> - <method name> - <run name>

File Edit View Utilities Options Window Help

New Edit New Run Browser Report Manual Setup Protocol Run Notes PostRun Log Settings Edit Cut Copy Paste Delete Bio-Rad Web

Add Step [V]

Isocratic Flow [Icon]

Load/Inject Sample [Icon]

Linear Gradient [Icon]

Change Valve [Icon]

Column Switching [Icon]

Repeat Steps [Icon]

Hold [Icon]

Pause [Icon]

Alarm [Icon]

Zero Baseline [Icon]

EGP [Icon]

Fraction Collection [Icon]

1	0.0	Isocratic Flow	Buffer A	100%	Volume: 5.0 ml
			Buffer B	0%	Flow: 1.00 ml/min
2	5.0	Change Valve	Change Flow Valve	Position: Forward Flow	
3	5.0	Load/Inject Sample	Load: Sample	Volume: 1.0 ml	
			Static Loop	Auto Inject Valve	Flow: 2.00 ml/min
4	6.0	Isocratic Flow	Buffer A	100%	Volume: 5.0 ml
			Buffer B	0%	Flow: 1.00 ml/min
5	11.0	Change Valve	Change Flow Valve	Position: Reverse Flow	
6	11.0	Linear Gradient	Buffer A	100% --> 30%	Volume: 3.0 ml
			Buffer B	0% --> 70%	Flow: 1.00 ml/min
7	14.0	Isocratic Flow	Buffer A	0%	Volume: 1.0 ml
			Buffer B	100%	Flow: 1.00 ml/min
8	15.0	Isocratic Flow	Buffer A	100%	Volume: 5.0 ml
			Buffer B	0%	Flow: 1.00 ml/min
9	20.0	Change Valve	Change Flow Valve	Position: Forward Flow	
	20.0	End of Protocol			

QuadTec	WL1 - 280nm	WL2 - 260nm	WL3 - 214nm	WL4 - 405nm	Econo Gradient Pump	Flow Rate	EGP %B	% Split
	0.40	0.15	2.00	0.00		0.00 ml/min	0 %B	0%
Maximizer Gradient Pump: F10			UV	Conductivity		SIM1/SIG ▼	SIM1/pH ▼	
1.00ml/min	0 %B	438 psi	1.003 AU	1.23 mS/cm		0.548 Volt	7.00 pH	

Figure 10-2. Programming for Reverse Flow Chromatography

11.0 MAINTENANCE

The BioLogic DuoFlow system requires minimal maintenance to assure reliable operation.

Note: Maintenance of the QuadTec UV/Vis detector and the Econo Gradient Pump (EGP) is discussed in their separate documentation.

11.1 CARE OF THE OUTER SURFACES OF THE INSTRUMENTS

During normal operation, spills and splashes may cause residues to form on the component cases.

To clean the case of an instrument, first unplug the instrument. Use a damp cloth to wipe down the outer case. Avoid wetting the power switch located below the front panel and the connectors on the rear of the unit.

The system rack tray should be mopped up and any residues rinsed away with water via the drain.

11.2 STORAGE OF THE DUOFLOW SYSTEM

For **overnight** storage, please note the following:

1. The system tolerates low salt buffer overnight, but sample and high salt buffers need to be washed out of the tubing.
2. Use deionized (DI) water and the 10mL syringe provided in the Fittings kit to wash behind each pumphead with at least 10 ml of water. This applies to both the F10 and F40 pumps.
3. If the system is in a cold environment, keep it powered on to prevent condensation. If it is at room temperature, it may be left off or on. (When it is at room temperature, the DuoFlow Controller (computer and monitor) can be turned off, to conserve energy and prolong monitor life.)
4. Thoroughly flush all valves with water, especially the Maximizer valves and the SVT3-2 and SV5-4 solenoid valves.

For **long-term** storage, please observe the following precautions:

1. Follow the column manufacturer's instructions to clean and store the column. Remove the column from inline.
2. Run deionized (DI) water through the entire system. Be sure to wash all wetted parts of both the Maximizer valves and the solenoid and automated valves to remove any contaminants.
Note: It is particularly important to wash out the Maximizer valves and the SVT3-2 and SV5-4 valves. Failure to do so may result in valve failure.
3. Use deionized (DI) water and the 10 ml syringe provided in the Fittings kit to wash behind each pumphead with at least 10 ml of water. This applies to both the F10 and F40 pumps.
4. Fill the entire system with either 20% ethanol (EtOH) or 0.05% sodium azide to inhibit bacterial growth. If necessary, fit the pump inlets, UV detectors and conductivity monitors, and all solenoid valves with 1/4-28 plugs which are provided in the Fittings kit.

11.3 CARE AND MAINTENANCE OF THE WORKSTATION PUMPS

The following three sections describe the procedures for system priming and periodic maintenance of the Workstation pumps.

11.3.1 Priming the Workstation Pumps and Removing Trapped Air Bubbles

Warning: Do not run the Workstation pumps dry (without buffers in line), as this may result in damage to the pumpheads.

Pumping 200 ml of 100% methanol (MeOH) through each pumphead at 5 ml/min greatly reduces air bubbles. Immerse inlet lines from both pumps into the methanol and pump at 50% B with the 40 psi backpressure regulator in place.

1. Check that both of the inlet tubings are securely placed into buffer bottles A and B. Inlet tubing should be finger-tightened. Inlet filters are highly recommended.
2. Remove air from inlet buffer lines and prime the Workstation pump.

Note: Air bubbles trapped behind the pumphead pistons will not damage the pumphead but will produce an erratic flow. This problem is easily diagnosed by watching the pressure readout on the BioLogic software status bar. If it is extremely erratic and unstable, then suspect a trapped bubble and repeat the above steps.

Some pressure fluctuation ($\pm 10\%$) is normal due to the response of the pressure transducer over the available flow rate of the Workstation pump.

- a. Insert a 10 ml syringe into the luer fitting of the priming port (see Figure 4-2) for Pump A of the F10 or F40 pumps. Open the priming valve by rotating it counterclockwise. Draw buffer into the inlet buffer line and into the syringe. Close the priming valve (rotate clockwise) prior to removing the syringe, and expel the buffer into a waste container.
- b. Repeat several times until no more bubbles are seen and then fill the syringe with 10 ml of buffer. Close the priming valve and remove air from the syringe by tapping the sides of the syringe to bring air bubbles to the top.
- c. Remove the Pump A outlet tubing (See Figure 4-2). Attach the syringe filled with buffer A to the priming port and open the priming valve. Cover the pump A outlet with a paper towel to catch expelled buffer. Pulse the syringe plunger vigorously to expel any bubbles trapped behind the pumphead piston. Do not push all of the buffer into the pump because air might be introduced in the last milliliter of buffer. Tighten the outlet fitting and close the priming port while the syringe is still in place.
- d. Repeat for Pump B.

11.3.2 Daily Maintenance

The pumpheads should be washed daily with water when high salt buffers are used. This can be automated through the use of an SVT3-2 diverter valve connected between the high salt buffer (buffer B) and pumphead B. Use one of the valve inlet ports to run the buffer and use the other inlet port to run water through the pumphead. Refer to Chapter 4, Figure 4-9. If the system is configured with a Maximizer (non-blending mode) use the B1 and B2 inlet valves to run water (B1) or buffer (B2) through the pumphead.

Washing behind the piston seal extends seal life. Insert a 10 ml syringe filled with deionized water in the hole at the top of the pumphead. A 10 ml syringe is provided in the Fittings kit. Each pumphead should get a 10 ml washout at the end of the day's operation. Run off water exits through the washout drain between the two pumpheads. It can be collected in a small beaker.

11.3.3 Routine Maintenance of the Workstation Pumps

The Workstation pumps require minimal maintenance to stay in good working condition. The DuoFlow F10 Pump and F40 Pump Maintenance kits include the piston seals, check valves, and O-rings for routine maintenance of the pumps. Indications that maintenance is required include flow rates that are below the specified rate, an erratic delivery of liquid, liquid running out of the washout drain during normal operation (often seen as salt crystal accumulation), and poor gradient performance.

Safety: Make sure that any hazardous material has been flushed from the system, the pumps are not running, and any residual pressure has been bled from the system. Disconnect the Workstation power cord. Wear eye protection, gloves, and other protection as appropriate.

1. Replacing a piston seal. (Refer to Figures 11-1 and 11-2.)

Each pump consists of PEEK pump blocks and screws, piston seals, O-rings and check valves. This procedure will require new piston seals. See Appendix D, Ordering Information.

- a. Soak the piston seals in 50% methanol to thoroughly wet them; sonicate in 50% methanol, if possible.
- b. Disconnect all tubing from the pumphead.
- c. Using the pumphead disassembly Allen wrench (or any standard 9/64" Allen wrench), remove the four long screws on the face of the pumphead center block (see Figure 11-1). Pull the pumphead away from the Workstation.
- d. Note that there are two piston assemblies per pumphead (see Figure 11-2). Each piston assembly consists of a piston, piston guide, and spring. Use a lab tissue to avoid getting fingerprints or oils onto the piston assembly. Carefully remove each piston assembly from its piston well in the pumphead assembly and set it aside.

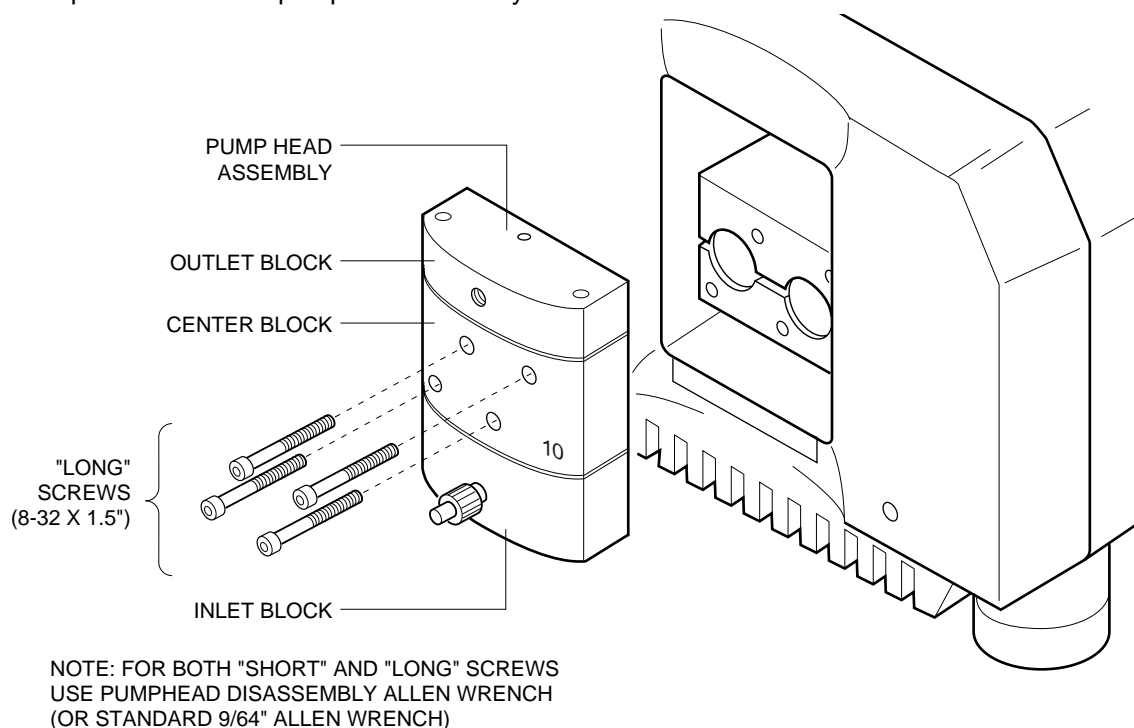


Figure 11-1. Workstation Pump Mechanism Parts

- e. Note the piston seal in each piston well. Always replace both seals at the same time. Using the piston seal removal tool, pry the seal from the piston well. To do this, you insert the tip of the seal removal tool into the seal and gently pry the seal away. Once a piston seal is removed, it cannot be reused.

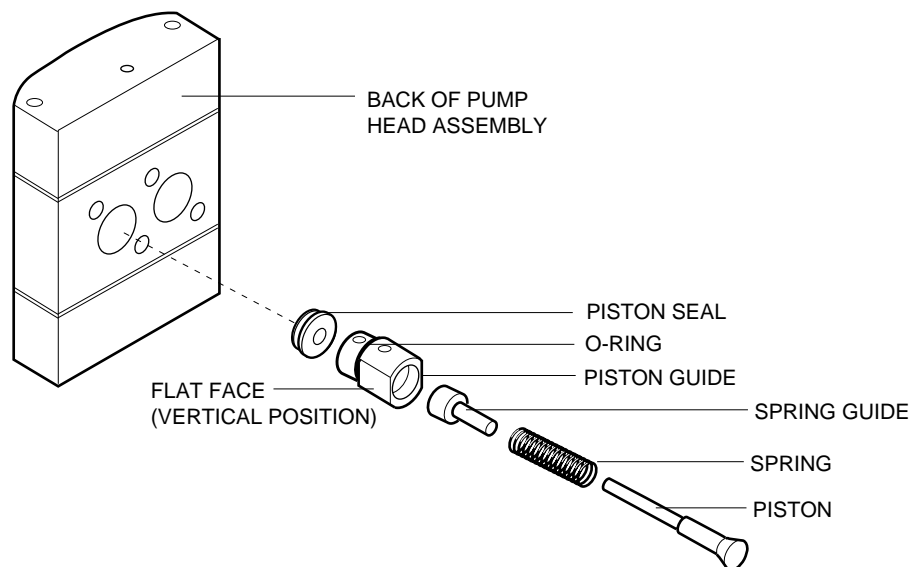


Figure 11-2. Piston Assembly and Access to the Piston Seal (view showing the back of the Pumphead Module)

- f. Using your finger, press each new piston seal into its recess. Use the piston guide to press the seal completely into its recess.
- g. Wipe the pistons with a dampened cloth before returning them to the piston wells. This ensures that no crystallized salt residues are present which could damage the new seals.

When returning each piston assembly to its piston well, make sure that the seals in the piston wells on the pumphead assembly are securely mounted in place. The piston assemblies should be oriented so that the flat faces on the piston guides are in the vertical position.
- h. Using the four long mounting screws removed earlier, remount the pumphead module to the Workstation so that it is secure. Insert and tighten the mounting screws. **Do not over tighten.**
- i. Condition the seal by pumping 100 ml of 100% methanol through each pumphead with the 40 psi backpressure regulator in place.
- l. Flush the system with water, and then fill and flush with the buffers to be used for the separation.

2. Cleaning and replacing a check valve.

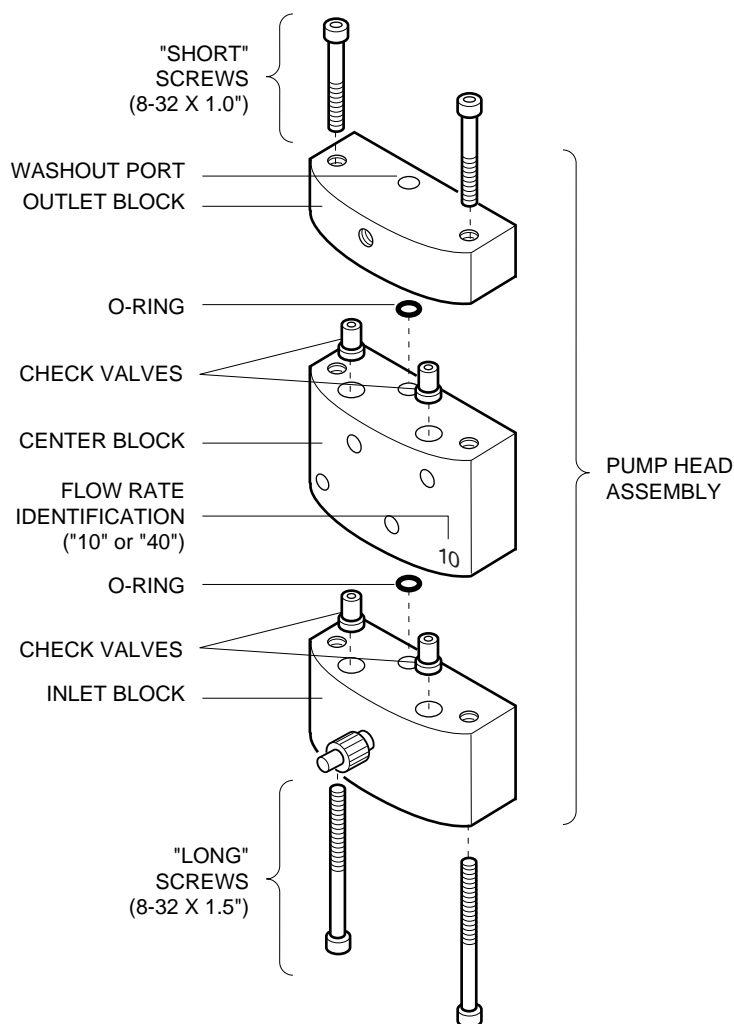
- a. Soak the new check valves in 50% methanol to wet them. Sonicate in 50% methanol, if possible.
- b. Remove the two short screws at the top of the pumphead assembly and the two long screws at the bottom of the pumphead assembly. Each pump separates into three blocks, revealing four check valves and two O-rings.
- c. Replace the check valves. It is not necessary to replace the O-rings, as they are not subjected to high pressure.
- d. Reassemble the pumphead. (Refer to Figure 11-3.) Check valves fit into the pumphead module in one direction only.
- e. Wipe the pistons with lab tissue before returning them to the piston wells, and rinse the piston guides in running water.

The piston assemblies should be oriented so that the flat faces on the piston guides are in the vertical position.

- f. Using the four long mounting screws removed earlier, remount the pumphead assembly to the Workstation so that it is secure. Insert and alternately tighten the mounting screws. Do not over tighten.
- g. Using the priming port on the front of the pumphead, purge the pump with 50% methanol and then flush with water.

3. Care of the Pistons.

To maintain pump performance, the pumphead should be rinsed on a nightly basis. The pump may be rinsed automatically by programming a water rinse step at the end of each protocol. It is also important to rinse behind the piston seals by injecting water through the washout port, located at the top of the pumphead. A standard syringe may be used for this purpose.



NOTE: FOR BOTH "SHORT" AND "LONG" SCREWS
USE PUMPHEAD DISASSEMBLY ALLEN WRENCH
(OR STANDARD 9/64" ALLEN WRENCH)

Figure 11-3. Pumphead Assembly

11.4 MAINTENANCE OF THE UV DETECTOR AND THE CONDUCTIVITY FLOW CELL

The following two sections discuss the cleaning of the UV detector and the Conductivity flow cell and replacement of the lamp in the UV detector.

11.4.1 Cleaning the UV Detector and the Conductivity Flow Cell

Cleaning of the UV flow cell is indicated when the baseline is very noisy or unstable yet air bubbles have been removed from the flow cell. However, problems with UV detection during medium pressure chromatography are often solved by a few simple precautions. Air bubbles trapped in the flow cells give rise to a “sawtooth” signal pattern that will never stabilize. We recommend always running with a backpressure regulator after the detector (if using low pressure columns, place the backpressure regulator between the pump outlet and mixer). Equilibrate the column for at least 30 minutes to allow all bubbles to clear. Always degass buffers before use.

Make sure all fittings are tight to prevent leaks. If liquid leaks down between the flow cell and the optics module case, the cell should be removed and dried with a stream of pure nitrogen (never use compressed air). The case should be dried using a warm air blower or left for several hours to completely dry.

Air bubbles trapped in the Workstation pump give rise to erratic flow rates that are sometimes interpreted as problems with the UV detector. Refer to the previous section.

If cleaning of the UV detector and the Conductivity flow cell is desired, note the following:

- **Filters:** The standard filter tray contains both a 280 nm and a 254 nm filter. To clean the filters, loosen the filter's thumbscrew and lift out the filter holder. The filters should be cleaned only when necessary using a dry lens tissue. To remove greasy fingerprints, use dilute ethanol or isopropanol solutions.
- **UV Flow Cell:** To remove the flow cell, undo the flat bottom fittings at the top and bottom of the flow cell. Then loosen the thumbscrews and push out the flow cell from the bottom. The flow cell is keyed to mount in one way only, so that it cannot be incorrectly mounted. Avoid touching the quartz windows with fingers.

If the flow cell is visually dirty, it should be cleaned with one or more of the following solvents: dilute sodium dodecyl sulfate (SDS), 1M hydrochloric acid (HCl), 1M sodium hydroxide (NaOH), ethanol, or acetone. Run the solution through the flow cell using a syringe and leave for no more than 5 minutes. Rinse extensively with water and then blow dry using a gentle stream of pure nitrogen. **Never** dry with compressed air from a “house” line as this will contain microdroplets of oil that will coat the cell.

When the detector is not in use, disconnect the flow cell and use a syringe filled with distilled water to clean out traces of salts and protein.

Before storing the flow cell, inject a dilute solution (10% to 25%) of ethanol or isopropanol into the cell to prevent microbial growth. Use the plugs provided with the flow cell to seal the flow cell's inlet and outlet lines.

- The Conductivity flow cell requires little maintenance other than rinsing routinely with deionized water.

11.4.2 Replacing the Lamp in the UV Detector

The following information covers the replacement procedures both for a new mercury lamp unit and a new zinc lamp unit. Note that the zinc lamp assembly has cooling fins and is larger than the mercury lamp assembly.

Indications that a lamp replacement is necessary include an unstable baseline and a decreased response to a standard concentration of a test chromophore. In this latter case, it is advisable to ensure that the flow cell is clean and that the correct wavelength filter is chosen before replacing the lamp. Refer to the previous section.

Note: As indicated during the following procedure, make sure the DuoFlow Workstation is turned off. This will eliminate the risk of electric shock, thermal burns, or UV light exposure to you or damage to the detector during cable connection.

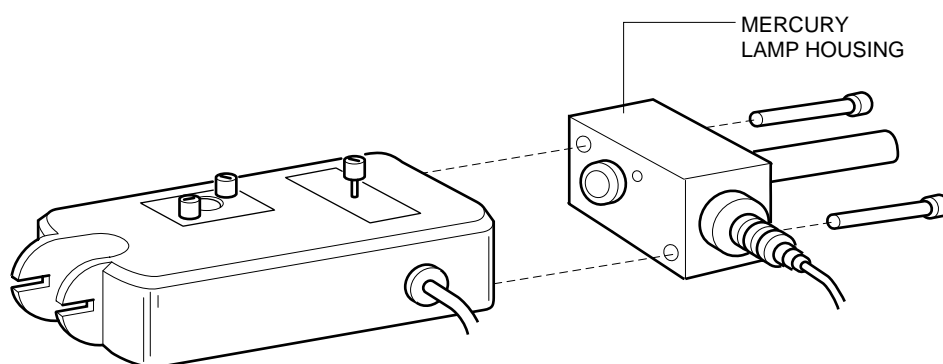


Figure 11-4. Replacing a Mercury Lamp in a Model OM-II UV Detector

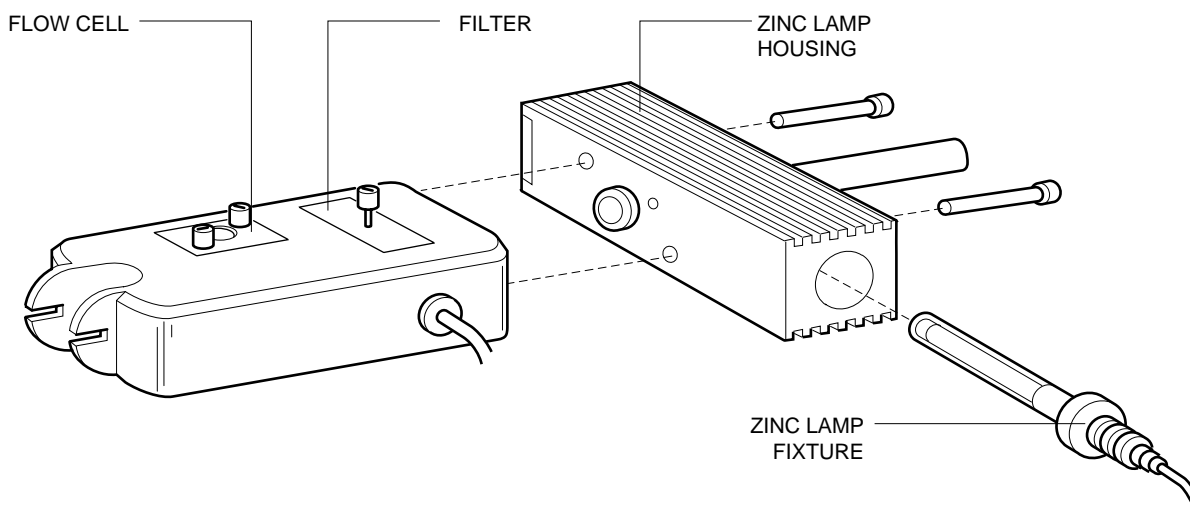


Figure 11-5. Replacing a Zinc Lamp in a Model OM-II UV Detector

1. At the DuoFlow Controller go to the Manual screen and turn off the lamp. Turn off power to the Workstation and unplug the UV Lamp cable, which is plugged into the UV Lamp connector on the rear of the Workstation.
2. Without disconnecting the lamp housing from the UV bench, undo the two small Allen screws securing the lamp fixture and gently pull the lamp out of the housing.
3. Remove the lamp unit from its packing material.
WARNING: Do not touch the glass part of the lamp. Oils from you fingers will degrade the lamp over a period of time.
4. Carefully insert the replacement lamp fixture. The replacement lamp can be installed only in one orientation due to a small guide which fits into the housing. Secure it using the Allen screws for that purpose. Note that extra screws are provided with the kit.

Note: Check that the O-ring seal at the lamp housing aperture is present and undamaged. If there is any indication of damage, or if it is missing, be sure to replace it using the O-ring supplied with the kit.
5. Reconnect the UV Lamp cable to the Workstation.
6. Turn on electrical power to the Workstation and at the DuoFlow Controller go to the Manual screen and turn on the lamp. Allow at least 30 minutes for the mercury lamp to warm up and at least several hours for the zinc lamp.

11.5 MIXERS

The Maximizer mixer and the MX-1 mixer may be disassembled for cleaning or to change the mixer capacity to accommodate the flow rate. Cleaning may be required if there is erratic gradient performance.

Both mixers consist of the mixer base and barrel, magnetic stir bar, screws, mixer top, and sealing O-rings.

Note: If the system has been used, make sure that any hazardous material has been flushed from the system, the pumps are not running, and any residual pressure has been bled from the system. Drain fluid from the mixer, disconnect any plumbing connections, disconnect the cable from the workstation, and then remove the mixer from the system rack.

1. Use the hex key provided (or any standard 5/32" hex key) to remove the four assembly screws from the top of the mixer.
2. Remove the mixer top and turn it upside down to remove the O-ring. If the O-ring does not easily dislodge, then use your fingers to remove it. Note the position of the O-rings (refer to Figure 11-6).
3. Wipe the inside of the mixer body with a paper towel. To clean the mixer barrel or mixer barrel extension, the O-rings, and the magnetic stir bar, soak or sonicate in a bath containing a mild detergent. Replace damaged O-rings.
Note: The stir bar is magnetized. To remove it, turn the mixer base upside down and tap it on your hand.
4. Reassemble the mixer. To re-insert the magnetic stir bar, place it on a tabletop and carefully place the mixer base over it.
5. Insert a 1/4-28 plug into one inlet and a 10 ml syringe into the other inlet port and flush the system with DI water or mild detergent solution. Reverse the location of the plug and syringe and repeat.

4. Refer to Figure 11-6 for re-assembling the mixer. Make sure that the magnetic stir bar lies flat and that the O-ring groove in either the mixer barrel or the mixer barrel extender faces up. Place an O-ring in each O-ring groove. If you are using only the mixer body, then only one O-ring is required. If either the mixer barrel or the mixer barrel extender is used, then two O-rings are required.

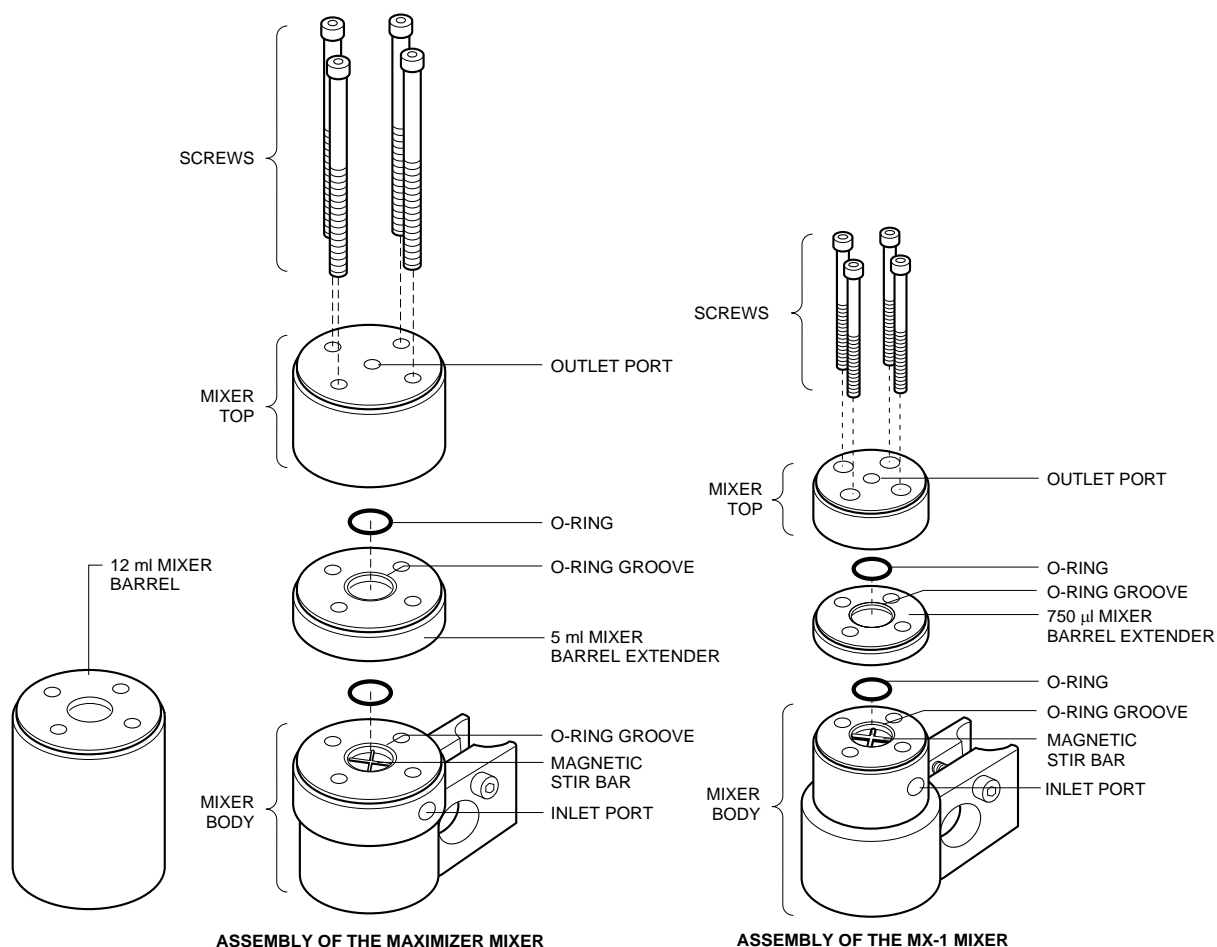


Figure 11-6. Mixer Assembly

11.6 VALVES

The following sections discuss the procedures for cleaning and repair of the SVT3-2, AVR7-3 and AVR9-8 valves.

11.6.1 SVT3-2 Diverter Valve

The SVT3-2 can be disassembled for cleaning or to replace parts which may become damaged over time. Indications of damage include valve leaks and valve switching problems. Items labeled in bold in Figure 11-7 are included in the SVT3-2 Valve Rebuild kit, catalog #760-0411. You will need a #1 Phillips (cross-head) screwdriver and a #0 Phillips (cross-head) screwdriver.

Note: These parts are rather small. Be sure you are working on a clean tabletop with room to lay out these parts and that your hands are clean.

Safety Note: Before disassembling the valve, be sure to flush any hazardous material from the valve and to disconnect it from the Workstation. Wear protective clothing, as appropriate.

To disassemble the valve, follow the procedure below:

1. Remove the large screw that attaches the clamp to the rack.
2. Use the #1 Phillips (cross-head) screwdriver to remove the two screws that attach the clamp to the valve. Set the clamp and screws aside.

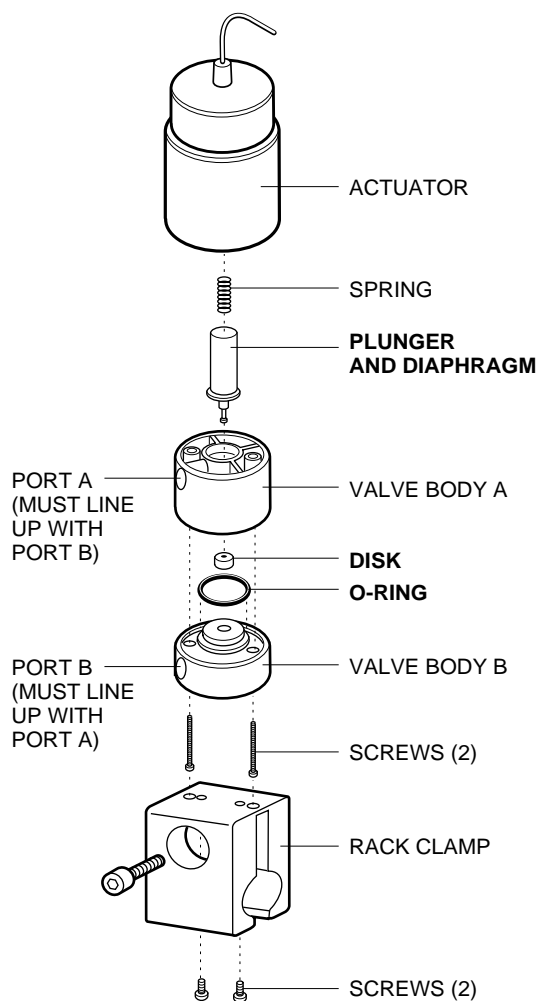


Figure 11-7. SVT3-2 Valve Assembly

3. Use the #0 Phillips (cross-head) screwdriver to remove the two screws and washers that secure valve bodies A and B to the actuator. Set the actuator aside, taking care not to lose the spring.
4. Use your fingers to remove the plunger/diaphragm assembly from the valve body.
5. Remove the label. A new label is provided in the rebuild kit. Note that ports A and B are on one side; the pump/common port is on the other side of valve body A. When reassembled, the new label will not fit properly if ports A & B are not adjacent to each other.
6. Carefully separate the two valve bodies. Note the location of the O-ring.
7. Inspect the disassembled valve. Make sure there are no scratches or foreign material on the sealing surfaces of the two valve bodies. Clean the valve bodies, if necessary, by soaking or sonicating in a bath containing a mild detergent. Replace all required parts, if necessary.

To reassemble the valve:

1. Insert the plunger/diaphragm assembly into valve body A. Invert it and install the sealing disk onto the plunger tip. Make sure the disk inserts into the plunger tip.
2. Install the O-ring on valve body B. Replacing the O-ring is easily done with your finger.
3. Put the two valve bodies together so that the two ports are next to each other. The two valve bodies must lie flush against each other, with no space in between them. Place the two larger screws (removed in step 3 above) through the valve assembly. Set this aside.
4. Set the actuator with its open end facing up.
5. Place the spring in the opening at the center of the actuator so that it fits into the deepest part of the opening. This may take a couple of tries.
6. Place the valve body assembly onto the actuator (the orientation is not specific), then tighten the two screws.
7. Attach the clamp to the valve body with the remaining screws.
8. Apply the valve label. Make sure that the hole in the label matches with the Pump/Common port and does not overlap it.

11.6.2 AVR7-3 and AVR9-8 Valves

The AVR7-3 and AVR9-8 valves are disassembled and cleaned in the same way. The replaceable parts in the valve are the rotor seal and the stator face assembly; these items are included in the AVR7-3 Rebuild kit (catalog #760-0401) and the AVR9-8 Rebuild kit (catalog #760-0403). Indications of damage include valve leaks and valve switching problems.

Safety Note: Before disassembling the valve, be sure to flush any hazardous material from the valve and to disconnect it from the Workstation. Wear protective clothing, as appropriate.

To disassemble the valve, follow the procedure below:

1. With the hex key that is provided, remove the three stator screws.
2. Lift the stator body and stator face assembly from the stator ring. (The stator face assembly usually stays with the stator body when it is lifted off.)

3. Remove the stator face assembly with your fingers and set it aside.
4. Remove the rotor seal by placing a small flat blade screwdriver into the slot on the side of the stator ring. Gently pry up the rotor seal, which will pop off.
5. Inspect the components from the disassembled valve. Make sure there are no scratches or foreign material on the sealing surfaces of the valve. Clean as necessary. Soak or sonicate in a bath containing a mild detergent.

To reassemble the valve:

1. Place the new rotor seal (slot-side facing the stator body), onto the three seal pins of the stator ring. It will only fit one way. Press the rotor seal so that it fits firmly.
2. Place the new stator face assembly onto the stator body so that the pins slip into the mating holes in the stator body. It will only fit one way.
3. Replace the stator body and stator face assembly so that the stator ring enters the mating hole on the stator body.
4. Using the three screws, re-assemble the valve. Tighten each screw equally.

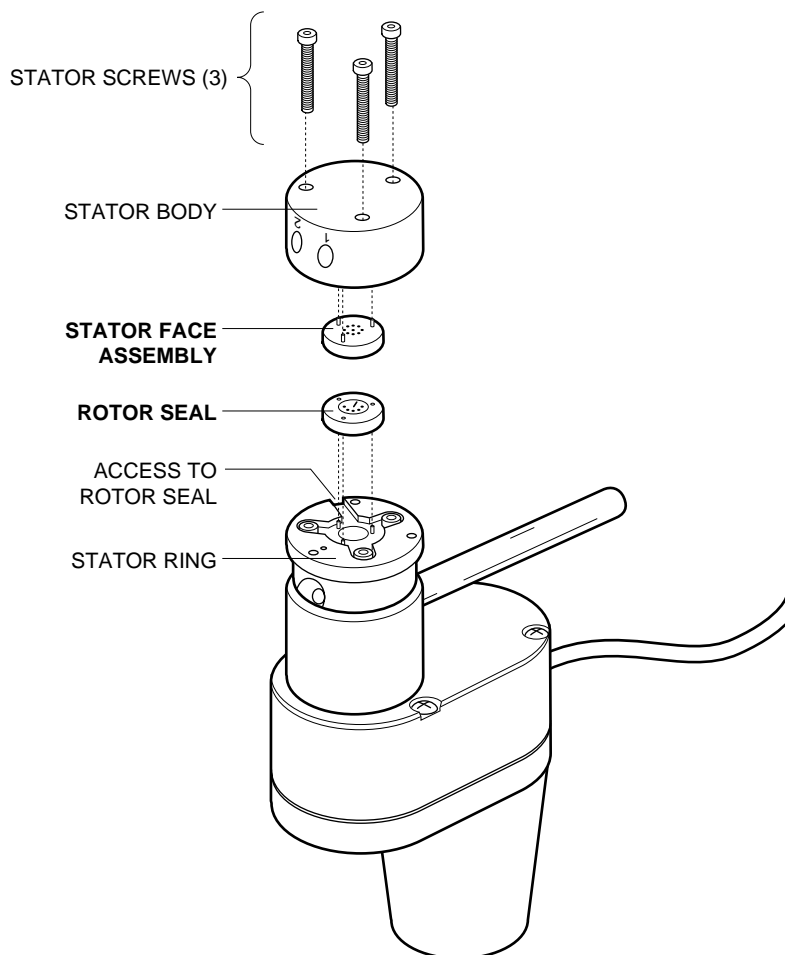


Figure 11-8. AVR7-3 and AVR9-8 Valve Assembly

11.7 MAXIMIZER VALVES

Over time, the Maximizer valves may become worn and require replacement. Indications of valve damage include valve leaks and valve switching problems. To replace a valve, you'll need a standard 9/64" hex key; one has been provided for this purpose.

Safety Note: Before removing a valve, be sure to flush any hazardous material from the valve. Wear protective clothing, as appropriate.

To remove the valve, follow the procedure below and refer to Figure 11-9:

1. Use the 9/64" hex key to remove the two screws that attach the valve to the Maximizer. Set the screws aside.
2. Lower the front of the valve to disengage the outlet tubing from the top of the valve.
3. Slowly pull away the valve to expose the wiring behind the valve.
4. Using your fingers, press the connector latch to separate the valve wiring from the Maximizer wiring.

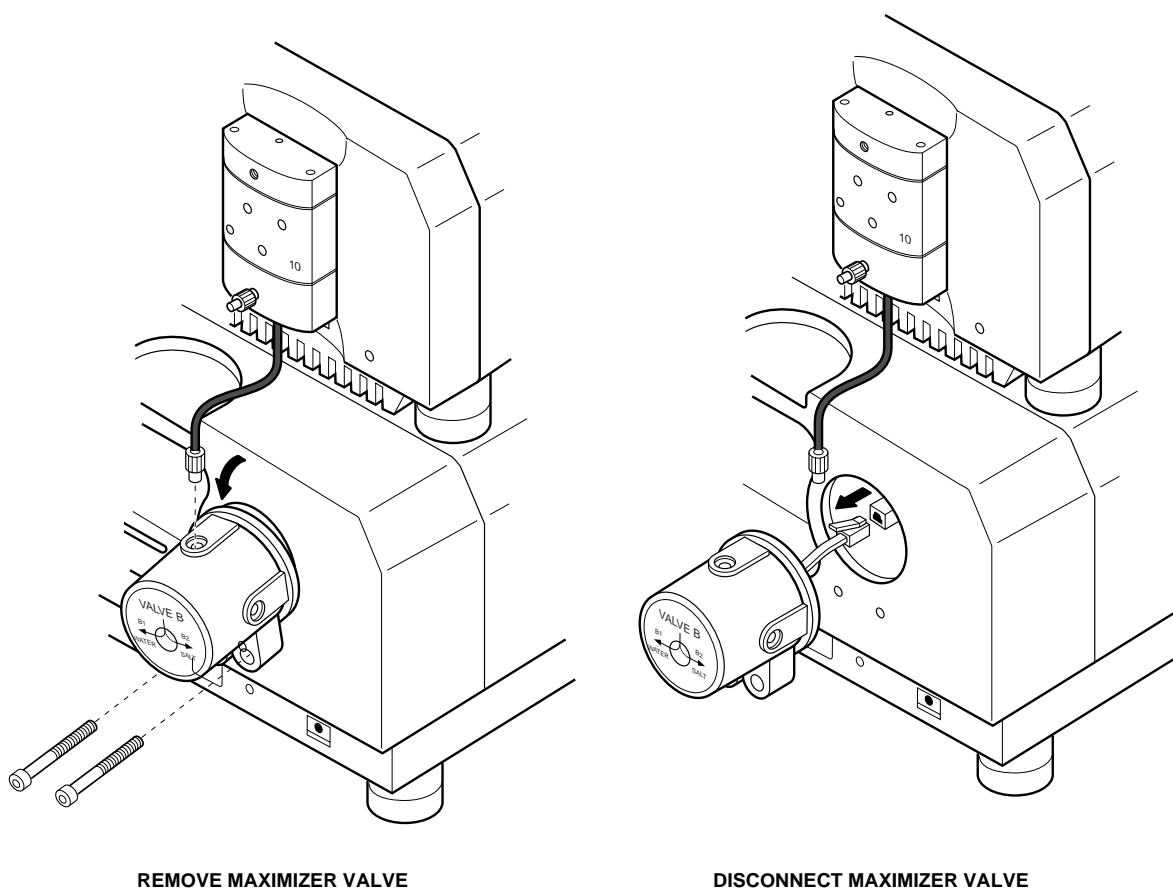


Figure 11-9. Replacing a Maximizer Valve

To attach the new valve, follow the procedure below:

1. Connect the valve wiring to the Maximizer wiring.
2. Place the valve against the Maximizer, but **do not** reattach the screws at this time. Rotate the valve slightly downward until you can engage the valve with the output tubing to the Workstation. Reattach the output tubing using its fittings.
3. Reattach the valve mounting screws.

12.0 TROUBLESHOOTING DUOFLOW SYSTEMS

The following section lists potential problems and some suggested solutions for the different instruments in the system. You can obtain more information about your system by registering for Consult Bio-Rad at www.biorad.com, where an online technical support service offers an extensive database of frequently asked questions (FAQs).

12.1 TROUBLESHOOTING THE DUOFLOW CONTROLLER AND SOFTWARE

Problem	Possible Cause	Solution
Software response is slow.	Database size may need to be reduced.	<ol style="list-style-type: none"> 1. Archive unnecessary methods and runs. <ol style="list-style-type: none"> a. Open the Browser window. b. Use COPY OUT to archive seldom used methods and runs. You may archive to a floppy disk, the BioLogic "C" drive, or any other drive selectable from the Drives drop down menu. 2. Use the Windows 2000 Disk Defragmenter utility. (Minimize the Duo-Flow application, and then from the Windows Start button, select Programs>Accessories>System Tools>Disk Defragmenter. Follow the online instructions to defragment files on your disk.
A method or run you expect is not displayed in the Browser.	There may be no apparent cause for this. Use the Reset button in the Browser toolbar.	<ol style="list-style-type: none"> 1. From the Browser toolbar, click on the Reset button, and then check the database tree for your method or run. Note: Using Reset collapses the database tree.
While in offline mode, the offline window is not displayed.	The Windows® 2000 minimize button may have been selected.	<ol style="list-style-type: none"> 1. Use any of the following three means of activating the offline window: <ol style="list-style-type: none"> a. Use the Windows drop-down menu. b. Use the Windows® 2000 taskbar. c. Simultaneously hold down the Alt-Tab keys. 2. With the cursor over the DuoFlow offline button at the bottom of the screen, click on the right mouse button and from the menu that is displayed, select Restore.
Problems using Browser functions.	If you are using the right mouse button rather than the toolbar buttons, make sure you have first used the left mouse button to highlight the user, project, method, or run name.	<ol style="list-style-type: none"> 1. Using the left mouse button, click on a user, project, method, or run name. 2. Using the right mouse button, select a function from the drop-down menu that is displayed.

Problem	Possible Cause	Solution
Instrument face-plates (eg. pump, UV, fraction collector) “drop-off” the screen when in Manual mode.	A communication problem has occurred.	<ol style="list-style-type: none"> 1. Exit the DuoFlow application and turn off the Controller. Turn off the Workstation. 2. Make sure all Instrument Bus (phone jack style) connections are secure in all the instruments. Turn the Controller and Workstation back on. 3. If the problem persists, contact Bio-Rad Technical Service.
Controller monitor displays unusual colors or characters.	The monitor or the Controller video card may be defective and need replacement.	<ol style="list-style-type: none"> 1. Exit the DuoFlow application and restart the Controller. 2. If the problem persists, contact Bio-Rad Technical Service.
No sound from monitor speakers.	<p>Cables not connected properly.</p> <p>“Mute” switch is activated.</p>	<ol style="list-style-type: none"> 1. Consult the monitor instruction manual to ensure that the cables are correctly connected. 2. Press the “mute” button, which is located on the front of the monitor to turn on the speakers.
Controller displays “General Protection Fault” message or “locks up” during normal operation.	A communication problem has occurred.	<ol style="list-style-type: none"> 1. Exit the DuoFlow application and restart the Controller. 2. If the problem persists, contact Bio-Rad Technical Service.

12.2 TROUBLESHOOTING THE DUOFLOW WORKSTATION PUMP

Problem	Possible Cause	Solution
Pump is not delivering the correct flow rate.	The incorrect pumpheads may be mounted, or the BioLogic Configuration software utility may not have been run after the pumpheads were changed.	1. Make sure the correct pumpheads (labeled F10 or F40 on the lower-right corner of the center block) are mounted. Then run the BioLogic Configuration software utility and select the correct pumphead.
	There may be problems with the fittings or tubing sizes. It is important to use the wide bore 1/8" OD tubing for the pump inlets and from all pre-pump buffer selection valves to the buffer containers.	2. Check pumps A & B and pre-pump valves. <ol style="list-style-type: none"> Make sure the inlet lines into pumps A and B are tight. If pre-pump valves are used, e.g., for buffer selection, make sure all tubing connections are secure and any unused ports are plugged.
	The pump may not be receiving buffer.	3. Check that there is buffer flowing to the pump. <ol style="list-style-type: none"> Ensure that the pump inlet lines are immersed in buffer. Ensure that the buffer bottles are positioned at or above the level of the Workstation. Ensure all inlet fittings are secure. Ensure that any inlet filters are clean. Remove temporarily to test for plugged filters.
	There may be air bubbles trapped in the pumpheads, causing erratic liquid delivery. Note: Always degas buffers before use.	4. Re-prime the pumps and purge the lines. <ol style="list-style-type: none"> Re-prime the pumps and ensure that the priming port is shut tight. Make sure the Inject Valve is set to Purge position and purge all lines. Refer to the Maintenance section on how to prime the pump and remove trapped air bubbles. To minimize the problem of air bubbles, degas buffers by stirring vigorously under vacuum for approximately 20 minutes. Use a heavy-wall side-arm Erlenmeyer flask, as standard flasks may implode under vacuum.
	Check valves are fouled and should be replaced. The check valves should be replaced on a regular basis, depending on the usage and type of solution or sample pumped through the pumphead. Refer to the Maintenance section for more information on how to replace a check valve.	5. Clean (or replace) the check valves (see Section 11.3.3, Routine Maintenance of the Workstation Pumps.) <ol style="list-style-type: none"> Cleaning: Sonicate check valves in a warm detergent solution, rinse with DI water, and re-install. Replacing: Replace badly clogged or damaged check valves. Recalibrate the pumps after cleaning/replacing check valves. Select Gradient Pump Calibration from the Utilities drop-down menu.

Problem	Possible Cause	Solution
Liquid is continuously leaking from the pumphead washout drain trough.	The pump's piston seals are worn or damaged.	<ol style="list-style-type: none"> 1. Replace the piston seals on both pumpheads. Clean pistons and piston guides with water, followed by methanol as a precautionary maintenance step. 2. Recalibrate the pumps after changing seals by selecting Gradient Pump Calibration from the Utilities drop-down menu.
Erratic or reduced flow rate when pumping onto columns which produce low back-pressures (eg., Econo-Pac® cartridges or small, agarose gel affinity columns).	Columns packed with soft gels or large particles typically produce very low back-pressures when run at low flow rates.	<ol style="list-style-type: none"> 1. Insert the backpressure regulator between the pump and the injection valve. <p>CAUTION: Low-pressure columns may burst if the backpressure regulator is placed after the column.</p>
Delivery of liquid is erratic and the pressure readout on the Controller status bar is fluctuating more than 20%.	<p>Severe fluctuation in the back-pressure readout indicates an air bubble trapped in one or both of the pumpheads.</p> <p>Note: Always degas buffers before use.</p>	<ol style="list-style-type: none"> 1. Put the AVR7-3 valve into the purge position and purge the system by pressing the Purge buttons on the front panel of the Workstation. 2. Stop or Pause the pumps and re-prime. Loosen the top fitting on the pumphead and force any trapped air out of the pumphead by squirting buffer through the priming port using a syringe. Refer to the maintenance section on how to prime the Workstation pump and remove trapped air bubbles by using methanol. Always use degassed buffers. 3. To minimize this problem, use degassed buffers and solutions. Degas buffers by stirring vigorously under vacuum for approximately 20 minutes. Use a heavy-wall side-arm Erlenmeyer flask, as standard flasks may implode under vacuum.
	The pump's check valves may need cleaning or replacement.	<ol style="list-style-type: none"> 4. Clean (or replace) the check valves (see section 11.3.3, Routine Maintenance of the Workstation Pump.) <ol style="list-style-type: none"> a. Cleaning: Sonicate check valves in a warm detergent solution, rinse with deionized (DI) water, and re-install. Replacing: Replace badly clogged or damaged check valves. b. Recalibrate the pumps after cleaning/replacing check valves. Select Gradient Pump Calibration from the Utilities drop-down menu.

Problem	Possible Cause	Solution
Loud or unusual noise coming from the front of the workstation.	Residue buildup behind piston seals.	1. The pistons should be rinsed on a nightly basis. This may easily be done automatically by programming a water rinse step at the end of each protocol. This may also be done manually by washing the pistons with the syringe provided in the Fittings kit.
	Sticky pistons.	2. Follow the maintenance procedure to carefully remove and disassemble the pumpheads. Wash the pistons thoroughly with water to remove debris or salt crystals. If the piston seals have many hours of use, they should be replaced following the information in the maintenance section. After re-assembly of the pumpheads, pump 100 ml of 100% methanol (MeOH) through each pumphead at 5 ml/min with the 40 psi backpressure regulator in place.
The backpressure displayed on the Controller status bar drops to zero or is much lower than expected.	Flow rate may have been changed.	1. Confirm that the flow rate setting has not been changed.
	There may be a tubing or fitting leak.	2. Re-inspect all tubing connections, especially at the pump inlet.
	Pump seals may need replacement.	3. Replace pump seals. A build-up of crystallized buffer salts on the rear of the pumphead is a clear sign for seal replacement. (see Section 11.3.3, Routine Maintenance of the Workstation Pumps.)
	Check valves may need cleaning or replacement.	4. Clean or replace check valves. (see Section 11.3.3, Routine Maintenance of the Workstation Pumps.)
No pressure (psi) reading on Controller status bar or psi value always reads zero.	Pump may not be running, or inlet lines are not primed, or there is a problem in the buffer flow.	1. Check that the pumps are running, and that the inlet lines are primed and not pulled out of the buffer reservoirs. Check for leaking fittings. 2. Check that the buffer flow is going to the column and not to waste via the injection valve purge position.
	The “zero pump pressure” calibration routine may have been used while the system was under pressure.	3. Stop the pump and undo the line from the pump to the valve such that the pressure transducer is definitely at zero psi. Select Gradient Pump Calibration from the Utilities drop-down menu and perform a Zero psi

Problem	Possible Cause	Solution
Incorrect backpressure readings for a given column.	Flow rate may have been changed.	1. Check that the pump is set to deliver the correct flow rate.
	Column may need cleaning and/or a frit replacement.	2. Consult the column's instruction manual for cleaning procedure. Alternatively, the system tubing may have an obstruction, so inspect the tubing path.
	The pressure transducer calibration may be incorrect.	3. Stop the pump, momentarily loosen the pump outlet fitting to release pressure. Select Gradient Pump Calibration from the Utilities drop-down menu and perform a Zero psi procedure.
High backpressure is shutting down the pumps.	<p>Maximum pressure for the pumps is: F10: 3500 psi F40: 1000 psi.</p> <p>The pressure limit settings in the Manual screen (see Figure 7-2) have been set too low.</p> <p>Pressure increases are due to a build-up of particulate matter in the system, causing a resistance to flow. An increase in flow rate also increases the pressure, so be sure to check this setting.</p>	<p>1. Remove the column and run the pumps to determine if the high backpressure is due to the column or not. If the column is suspect, the column may need cleaning along with a frit replacement. Consult the column's instruction manual for cleaning procedure.</p> <p>2. Alternatively, the system tubing may have an obstruction, so inspect the tubing path. To isolate the blockage, start by loosening connection fittings at the detector and work backwards towards the column and pumphead.</p> <p>3. Prevent or minimize high backpressure problems by filtering buffers and samples and by changing the pump seals before they deteriorate completely.</p>

12.3 TROUBLESHOOTING THE UV DETECTOR AND UV TRACE

Problem	Possible Cause	Solution
<p>UV baseline is unstable or noisy.</p> <p>UV baseline shows a reproducible zig-zag or saw-tooth trace.</p>	<p>Air bubbles trapped in the analytical 5 mm Z cell are the most common cause of noisy UV baselines.</p> <p>Note: Always degas buffers before use.</p>	<ol style="list-style-type: none"> 1. Degas buffers by stirring vigorously under vacuum for approximately 20 minutes. Use a heavy-wall side-arm Erlenmeyer flask, as standard flasks may implode under vacuum. 2. Plumb the Z cell with the column outlet connected to the bottom of the flow cell. This will force air bubbles to rise to the top of the flow cell and dissipate. 3. Use of the 40 psi backpressure regulator supplied with the DuoFlow system will remove most air bubbles. Place it directly after the conductivity flow cell and run buffer for several minutes. <p>If the column being used cannot be run with the backpressure regulator and air bubbles are a continuing problem (for example, during some affinity chromatography steps), use the 2mm path length cuvette cell. The straight line flow of the cuvette cell makes bubble removal easier.</p>
Excessive pump pulsations exhibited as regular noise on the baseline.	Air bubbles trapped in the pumpheads can produce exaggerated pulsations which appear as a noisy baseline.	<ol style="list-style-type: none"> 1. Purge the pumpheads to remove the bubble. Refer to Section 11.3.1, Priming the Workstation Pump and Removing Trapped Air Bubbles.
Baseline noise continues when pumps are turned off.	Noise spikes can also be caused by external environmental influences.	<ol style="list-style-type: none"> 1. If the spikes occur at regular intervals (for example, once every 20-30 sec), check for the presence of heating baths, drying ovens, or other heating devices on the same electrical circuit or in close proximity to the DuoFlow system. Turn off these devices to see if the problem goes away.

Problem	Possible Cause	Solution
UV trace will not zero.	If no air bubbles are present in the flow cell, check that the cell itself is clean both internally and externally.	<ol style="list-style-type: none"> 1. Clean the interior of the cell by passing 1M sodium hydroxide (NaOH), water, methanol and water through the cell. Dry the interior of the cell with a stream of high purity nitrogen gas (never use "house" compressed air as it may contain oil droplets). 2. Check for leakage of liquid from the top of the optics unit onto the outside quartz windows of the flow cell. Clean the windows with a soft, non-abrasive damp cloth. 3. Condensation may occur on the exterior windows of the flow cell when moving the optics unit from the lab to a cold room and vice versa. Always allow an equilibration period to compensate for such temperature effects.
Drifting baseline.	Non-homogeneous eluant perhaps due to poor mixing or flow rate variation.	1. Check flow rates of both pumps; recalibrate if needed by selecting Gradient Pump Calibration from the Utilities drop-down menu.
	Slow column equilibration.	2. Certain ion-exchangers are slow to re-equilibrate especially if just sanitized. Allow a longer equilibration period.
	If drift is due to a higher absorbance, suspect that UV absorbing material may be leaching from the column.	3. To confirm this, remove the column and run deionized (DI) water or an aqueous non-absorbing buffer. If the baseline is stable, then the column is suspect. Consult the column's user manual for cleaning procedures.
Negative peaks.	The sample is applied to the column in a different buffer from that used to equilibrate and elute the column. Refractive index changes may be responsible for the negative peaks.	1. Apply the sample in the same buffer used to equilibrate the column.
	The elution buffer may have a higher UV absorbance than the sample components.	2. Check the elution buffer's UV absorbance.

Problem	Possible Cause	Solution
False or “ghost” peaks.	The injection valve, injection port and loop were not rinsed out between sample injections.	<ol style="list-style-type: none"> 1. It is a good idea to allow a larger volume of injection buffer to pass through the inject valve and loop. For example, if the sample loop contains 100 µl, then program a load/injection volume of 200 µl to completely flush the valve and loop. 2. Always clean large volume dynamic loops, such as the Bio-Rad DynaLoop, between use to avoid cross contamination.
	Strongly retained sample components may still be eluting from a previous run.	<ol style="list-style-type: none"> 3. Use stringent elution conditions or consult the column’s user manual for an appropriate wash step to remove such components between runs.

12.4 TROUBLESHOOTING THE CONDUCTIVITY FLOW CELL AND TRACE

Problem	Possible Cause	Solution
No values for buffer conductivity are displayed on the Controller status bar.	There may be a loose or incorrect cable connection.	<ol style="list-style-type: none"> 1. Make sure the cable from the Conductivity flow cell is plugged into the correct connector on the rear of the Workstation and that the pins are not bent.
	Conductivity monitor may need calibration.	<ol style="list-style-type: none"> 2. Use the Conductivity Flow Cell Constant Calibration function from the Utilities drop-down menu. Either input the constant value which is shown on the tag attached to the flow cell or calibrate the flow cell using 0.5M sodium chloride (NaCl). Note that the relationship between the conductivity reading and salt concentration is not linear. The curve will flatten out at higher salt concentrations. 3. If a Maximizer is connected, make sure the Conductivity monitor is plugged into it.
Conductivity trace does not follow exactly the theoretical gradient trace %B.		<ol style="list-style-type: none"> 1. The Conductivity flow cell is typically placed after the UV flow cell. The Conductivity trace will be offset from the theoretical gradient based upon the column and system volume.
Distorted conductivity trace.	Mixer capacity is incorrect	<ol style="list-style-type: none"> 1. Refer to Section 2.4, Mixers, for discussion of mixer capacities.

12.5 TROUBLESHOOTING THE MAXIMIZER BUFFER BLENDING

Problem	Possible Cause	Solution
No values for pH are displayed on the Controller status bar.	There may be a loose or incorrect cable connection.	1. Make sure the cable from the pH monitor is connected to a SIM or Maximizer and that the SIM is connected to the system bus.
Noisy or unstable readings	Air bubble on pH probe membrane	1. Remove air bubble.
Drift	Solution temperature is changing	1. Allow all solutions to come to thermal equilibrium.
Fluctuations observed in pH readings during run	Changes in salt concentrations during a run may cause errors in the observed pH	1. The actual pH is may not be in error but only the observed pH. Collect fractions and measure the pH of the solution using a suitable bench top pH monitor during a run. 2. Ensure that the mixer size is appropriate for the flow rate.
The observed buffer pH does not match the desired pH.	The software pH correction may have been entered incorrectly in the Buffer Blending Setup.	1. Check the pH correction setting and change it if necessary.
	The pH electrode is not calibrated.	2. Recalibrate the pH electrode using standards that span the pH range of the buffer being used. Buffers may be incorrectly prepared.
The pH electrode will not calibrate	The electrode may be dirty or damaged.	1. Clean the pH electrode as described in the instructions that came with the probe. Replace the pH electrode if necessary.

12.6 TROUBLESHOOTING OTHER BIO-RAD INSTRUMENTS AND DEVICES

For troubleshooting information about other Bio-Rad instruments and devices, such as the BioFrac fraction collector and the QuadTec detector, refer to their separate documentation.

APPENDIX A. SPECIFICATIONS

System Power Requirements: BioLogic DuoFlow Controller, Workstation, and Maximizer

- 100 V ~ 5.3 A
- 230 V ~ 3.5 A
- 50 - 60 Hz

DuoFlow Dell Controller

- Pentium III® Processor, 20 GB hard drive, 128 MB RAM
- SoundNIC and video 4MB graphics performance accelerator
- Dell M781 multimedia 16" monitor
- 3.5" floppy drive; CD-RW 8x CD-ROM drive
- Windows® 2000 operating system
- Microsoft® PS/2 IntelliMouse™
- DuoFlow software, version 4.0 or higher
- Networkable for printer and file access

Maximizer

- Size: 18.5" x 14.25" x 4.33"; 47 x 36 x 11 cm (L x W x H)
- Weight: 11 lbs; 4.8 kg
- Environmental: Cold room compatible
- Solvent compatibility: 1M sodium hydroxide (NaOH), hydrochloric acid (HCl), and organic acids; 0.1M sulfuric acid; 7M urea and guanide hydrochloric acid; 100% methanol (MeOH), isopropanol (IPA), and acetonitrile; 0.1% trifluoroacetic acid (TFA); 1% detergents (including sodium dodecyl sulfate (SDS)) and TritonX-100

Workstation pump

- Dual piston, positive displacement solvent pumps, with interchangeable solvent delivery pumpheads (F10 and F40) made of bio-compatible PEEK
- F10 pumpheads: 3,500 psi (233 bar, 23 MPa) from 0.01 to 10.0 ml/min in 0.01 ml/min increments. *With* Maximizer: 3,500 psi (233 bar, 23 MPa) from 0.5 to 20.0 ml/min in 0.02 ml/min increments.
Flow accuracy: 0.01 to 10.0 ml/min: 2%
Flow reproducibility: 0.01 rsd
- F40 pumpheads: 1,000 psi (66 bar, 6.6 MPa) from 0.5 to 40.0 ml/min in 0.01 ml/min increments. *With* Maximizer: 1,000 psi (66 bar, 6.6 MPa) from 1.0 to 80.0 ml/min in 0.02 ml/min increments
Flow accuracy: 0.5 to 40.0 ml/min: 2%
- Compositional accuracy: $\pm 1.0\%$
- High pressure dynamic mixing. MX-1 mixer volume without barrel is 263 μ l; mixer with volume expandable to 750 μ l and 2 ml with added extender barrels mixer barrel extender: 750 μ l; mixer with mixer barrel extender: 2 ml. Maximizer mixer volume without barrel: 750 μ l; mixer with mixer barrel extender: 5 ml; mixer with expansion barrel: 12 ml.
- Built-in pressure transducer
- Gradient by software control: 1% increments

USB Bitbus Communicator

- Size: 4.25" x 2.75" x 1.125"; 11 x 7 x 3 cm (L x W x H)
- Weight: 6 oz
- Electrical: 5 VDC output

General

- System fittings: Flangeless 1/4-28 flat-bottom fittings, fingertight nuts and ferrules
- System Tubing for flow rates up to 15 ml/min: 1/16" (1.6 mm) OD, 0.020" (0.51 mm) ID orange PEEK tubing. The approximate volume of 1 cm of tubing is 2 µl.
System Tubing for flow rates of 15 ml/min and greater: 1/16" (1.6 mm) OD, 0.030" (0.76 mm) ID green PEEK tubing. The approximate volume of 1 cm of tubing is 4.5 µl.
Pump Inlet Tubing: 1/8" (3.2 mm) OD, 0.062" (1.6 mm) ID Teflon tubing
- Wetted materials: 100% bio-compatible
- Solvent compatibility: 1.0 M sodium hydroxide, hydrochloric acid and organic acids, 1.0 M sulfuric acid, 7M urea and Guanidine hydrochloric acid, 100% ethanol, isopropanol, and acetonitrile, 0.1% trifluoroacetic acid (TFA), 1% detergents (including sodium dodecyl sulfate (SDS), Triton X-100)
- Sound level, Workstation: <70 dBa
- Dimensions, Workstation (approx.): ~35 x 41 x 18 cm (W x D x H); height with 3-tray rack: 76 cm
Dimensions, Maximizer (approx.): 36 x 47 x 11 cm (W x D x H)
- Weight (Controller, Workstation, monitor, rack, inject valve and detection modules): 34.5 kg (76 lbs) (approximate). Weight, Maximizer: 4.8 kg.
- Operating temperature (excluding Controller and USB Bitbus Communicator): 4° – 40°C

Detection

- Fixed wavelength, mercury lamp with 280/254 nm filter, auto zero; 0.0001 - 2.0 AUFS detection range
- Analytical (5 mm pathlength, 16 µl total volume) flow cell
- Preparative (2 mm pathlength 30 µl volume) flow cell
- Optional 214 nm detection kit (includes zinc lamp, housing, and 214 nm filter)
- Optional wavelength filters include: 214, 313, 365, 405, 436, and 546 nm
- Conductivity detection (500 mS/cm; 6 µl flow cell; accuracy ± 2% full scale)
- Optional flow-through pH monitor (includes flow cell, pH electrode, and SIM)
- Cold room compatible

Sample Loading

- Fixed sample loops, 100 µl to 5.0 ml; automated injection valve included
- Large volume loading through the Gradient Pump. Optional inlet select valves (SV5-4, AVR9-8) for sanitation and multiple sample use.
- Variable volume sample loading through the DynaLoop sliding-piston sample loop.
- Software control of an auxiliary pump for multiple sample loop fills and large volume dynamic loop filling.

Valve Control

- Up to three low-pressure, automated solenoid valves (SV5-4 and SVT3-2) for stream selection.
- Up to three high-pressure, automated valves (AVR7-3 and AVR9-8) for sample inject, column switching and stream selection.

Fraction Collection

- BioFrac fraction collector (collection by Collect All, Threshold, Collection Windows, Collection Windows + Threshold)
- Model 2110 fraction collector (collection by Collect All; when used with optional SVT3-2 Diverter valve, it also offers collection by Threshold, Collection Windows, Collection Windows + Threshold)

Chart Recorder Control

- Model 1327 Dual pen recorder. Paper feed Start/Stop, Pen Up/Down

Signal Import Module

- Up to two Signal Import Modules for importing analog signals from peripheral chromatography detectors

APPENDIX B. PRESSURE CONVERSION TABLE

Use this table to convert pressure units for the pump.

Table B-1
Pressure Conversion

Multiply the units in the left column by the conversion factors listed.

Pressure	PSI	atm	Kg/cm ²	Torr	kPa	Bar	Inches Hg
PSI	1	0.068	0.0703	51.713	6.8948	0.06895	2.0359
atm	14.696	1	1.0332	760	101.32	1.0133	29.921
Kg/cm²	14.223	0.9678	1	735.56	98.06	0.9806	28.958
Torr	0.0193	0.00132	0.00136	1	0.1330	0.00133	0.0394
kPa	0.1450	0.00987	0.0102	7.52	1	0.0100	0.2962
Bar	14.5038	0.9869	1.0197	751.88	100	1	29.5300
Inches Hg	0.4912	0.0334	0.0345	25.400	3.376	0.03376	1

Example 1: Convert 521 psi to Kg/cm²
(521 x 0.0703) or (521 psi = 36.63 Kg/cm²)

Example 2: Convert 60 Bar to psi
(60 x 14.5038) or (60 Bar = 870 psi)

Example 3: Convert 42.70 Bar to Kg/cm²
(42.70 x 1.0197) = (42.70 Bar = 43.54 Kg/cm²)

APPENDIX C. WARRANTY STATEMENT

Bio-Rad Laboratories warrants that every instrument it sells will be free from defects in materials and workmanship when it leaves the factory and that, if such defects appear within the first year following purchase, the defective part(s) will be replaced or the entire unit will be replaced, at Bio-Rad's option, free of any charges to the buyer other than expenses incurred in returning the unit to the factory. Bio-Rad's obligation under this warranty is specifically limited to the aforementioned replacement or repairs. However, the following defects are specifically excluded:

1. Defects caused by improper operation.
2. Repair or modification done by anyone other than Bio-Rad Laboratories or their authorized agent.
3. Use with fittings or other spare parts not specified by Bio-Rad Laboratories.
4. Damage caused by deliberate or accidental misuse.
5. Damage caused by disaster.
6. Damage due to use of improper solvent or sample.
7. Tubing, fittings, lamps, pistons, check valves, piston seals, and other consumables.

"The foregoing obligations are in lieu of all other obligations and liabilities including negligence and all warranties, of merchantability, fitness for a particular purpose or otherwise, expressed or implied in fact or by law, and state Bio-Rad's entire and exclusive liability and Buyer's exclusive remedy for any claims or damages in connection with the furnishing of goods or parts, their design, suitability for use, installation or operation, Bio-Rad will in no event be liable for any special, incidental or consequential damages whatsoever, and Bio-Rad's liability under no circumstances will exceed the contract price for the goods for which liability is claimed."

Under this warranty Bio-Rad's obligation with respect to transportation expenses is limited to the cost of shipping the repaired or replacement instrument to the buyer, provided that such repair or replacement comes within the terms of this warranty.

For additional help, contact your local Bio-Rad representative. In the United States, call Technical Service at 1-800-4BIORAD.

APPENDIX D ORDERING INFORMATION

- 760-0180 F40 Pump Kit. Includes fully assembled F40 pumpheads with check valves and seals installed, four F40 piston assemblies, one mixer barrel extender, two mixer O-rings, a 2mm UV flow cell, PEEK tubing 0.030" (0.76 mm) ID, and necessary tools.
- 760-0110 F10 Pump Kit. Includes fully assembled F10 pumpheads with check valves and seals installed and four F10 piston assemblies.
- 760-0164 F10 Pump Maintenance Kit, services one F10 pumphead. Kit includes two piston seals, four check valves, seal removal tool, and two O-rings.
- 760-0161 F10 Piston Seals (2) and Seal Tool. Two piston seals required to service one F10 pump.
- 760-0162 F10 Piston Assembly (2). Two piston assemblies required to service one F10 pump.
- 760-0184 F40 Pump Maintenance Kit, services one 40ml pump. Kit includes two piston seals, two check valves, and two pistons.
- 760-0172 F40 Piston Seals (2) and Seal Tool. Two piston seals required to service one F40 pump.
- 750-0162 Check Valve (1). Four check valves required to service one pump; for F10 and F40 pumps.
- 750-0111 EZ Logic Integration Software Package.
- 760-2030 USB Bitbus Communicator.

Mixers

- 760-0170 MX-1 Mixer. Mixer includes mixer body (263µl) and expansion barrel (750µl).
- 760-0171 MX-1 Mixer Barrel Extender (2ml).
- 100-1627 MX-1 Mixer Service Kit.
- 920-8529 MX-1 Mixer Barrel Extender (1) (750µl).
- 760-2010 Maximizer Mixer. Includes mixer body (750µl), 5ml and 12ml expansion barrels, five O-rings, stir bar, and installation screws for all barrel sizes.
- 760-2005 Maximizer Mixer Barrel Extender (5ml).
- 760-2012 Maximizer Mixer Barrel Extender (12ml).

Detectors

- 760-1300 QuadTec DuoFlow Detector Kit 100/110, 200/240 V.
Includes QuadTec Detector, 3mm flow cell, System Cable 25 QuadTec RS232 cable, 4x10-32 Fingertight fittings, Instrument Control Module (ICM), System Cable 26 ICM power cable, System Cable 17 instrument bus cable, QuadTec Instruction Manual, US power cord.
- 760-1330 QuadTec Deuterium Lamp.
- 760-1331 QuadTec Halogen Lamp and Holder. The holder is required for first time use.
- 760-1332 QuadTec Halogen Lamp.
- 760-1306 QuadTec Flow Cell, PEEK 3mm path length.
- 760-1311 QuadTec 4x10-32 Long fittings for 3mm flow cell.
- 760-1406 QuadTec Flow Cell, PEEK High speed for 80 ml/min flow rates.
- 760-1408 QuadTec 4x10-32 Long fittings for Hi speed flow cell.
- 760-1301 QuadTec Instruction Manual.
- 750-0200 UV Detector Kit. Includes UV Detector with 254/280nm filter, 5mm flow cell, conductivity flowcell, and backpressure regulator.
- 750-0214 214 nm Conversion Kit (Zinc lamp, housing, 214 nm filter) for Model OM-II UV Detector optics modules beginning with serial number 362 BR.
- 750-0215 214 nm Conversion Kit (Zinc lamp, housing, 214 nm filter) for Model OM-IO UV Detector optics modules, beginning with serial number 345 BR and prior to 362 BR.
- 750-0210 UV Detector Flow Cell, 2 mm path length.
- 750-0212 UV Detector Flow Cell, 5 mm path length.
- 750-0216 Mercury Lamp, replacement.
- 750-0217 Zinc Lamp (214 nm), replacement.
- 750-0220 Filter, 280 and 254 nm.
- 750-0221 Filter, 214 nm.

750-0223 Filter, 365 nm.
750-0224 Filter, 405 nm.
750-0225 Filter, 436 nm.
750-0226 Filter, 546 nm.
750-0240 Conductivity Flow Cell, replacement.
750-0230 Backpressure regulator.
750-0502 Signal Import Module (SIM).

Valves

760-2008 Maximizer Proportioning Valve.
760-0406 AVR7-3 Sample Injection Valve.
760-0401 AVR7-3 Valve Rebuild Kit.
750-0471 AVR7-3 Sample Injection Port.
760-0408 AVR9-8 Stream Select Valve.
760-0403 AVR9-8 Valve Rebuild Kit.
760-0410 SVT3-2 Diverter Valve.
760-0411 SVT3-2 Valve Rebuild Kit.
750-0415 SV5-4 Select Valve.
125-0224 Inject Needle, 22-gauge blunt, fits sample injection port.

750-0251 Rack

750-0268 Rack expansion kit (two trays, two vertical bars, sixteen sleeves).
750-0260 Column clamp set.
750-0261 Rack tray; includes eight sleeves, one drain/plug.
750-0262 Vertical bar, long (2).
750-0263 Vertical bar, short (2).
750-0264 Horizontal bar kit (two tie bars, four bar clamps).
750-0265 Bar clamps (5).
750-0266 Cable Manager clips (4).
750-0269 System wrench set.

pH Monitor

760-2040 DuoFlow pH Monitor.
760-2042 pH Electrode, replacement.
760-2044 Flow Cell, replacement.
750-0502 Signal Import Module (SIM).
910-1828 O-ring, pH Flow Cell, size 2-009.
910-3014 O-ring, pH Flow Cell, size 2-018.
760-2046 BioLogic pH Monitor Tubing Kit.

Sample Injection Loops

750-0490 Small Volume Sample Loop Kit (100, 250, and 500µl loops). Nominal pressure limit of 4000 psi.
750-0491 Large Volume Sample Loop Kit (1, 2, and 5ml loops). Nominal pressure limit of 3000 psi.
750-0492 100 µl injection loop. Nominal pressure limit of 4000 psi.
750-0493 250 µl injection loop. Nominal pressure limit of 4000 psi.
750-0494 500 µl injection loop. Nominal pressure limit of 4000 psi.
750-0495 1 ml injection loop. Nominal pressure limit of 3000 psi.
750-0496 2 ml injection loop. Nominal pressure limit of 3000 psi.
750-0497 5 ml injection loop. Nominal pressure limit of 3000 psi.
750-0451 DynaLoop 25, for 25ml samples
750-0452 DynaLoop 90, for 90ml samples

Fittings

760-0550	DuoFlow Fittings Kit.
750-0560	DuoFlow Fittings Tool.
750-0553	1/8" (3.2 mm) Pre-pump Fittings (includes nut, ferrule, and lock ring) (10).
750-0554	1/16" (1.6 mm) Post-pump Fittings (includes nut, ferrule and lock ring) (10).
750-0570	1/8" (3.2 mm) Nut (5).
750-0571	1/8" (3.2 mm) ferrule and lock ring (5).
750-0569	1/16" (1.6 mm) Nut (10).
750-0556	1/16" (1.6 mm) ferrule and lock ring (10).
750-0703	Inline Filter Kit. Includes one inline filter, two replacement Ultra High Molecular Weight PolyEthylene (UHMWPE) filter frits.
750-0704	Filter frits, replacement (5).
750-0566	Bottle Cap Kit, (includes two bottle caps, two cap plugs).
750-0559	Caps (5 pack).
750-0561	1/4-28 to M6 (FPLC column adapter) (2).
750-0562	1/4-28 to 1/4-28 union (5).
750-0563	1/4-28 plug (5).
750-0564	HPLC Column to DuoFlow System Fittings Kit (1 set).
750-0565	Econo-Column to DuoFlow System Fittings Kit (1 set).
732-0113	Econo-Pac® Cartridge to DuoFlow System Fittings Kit (1 set).
750-0471	Econo-Column® to DuoFlow System Fittings Kit (1 set).

Tubing

750-0603	Teflon tubing, 1/8" (3.2 mm) OD x 0.062" (1.6 mm) ID x 15 feet (4.6 m), for pre-pump connections.
760-0604	PEEK tubing, 1/16" (1.6 mm) OD x 0.020" (0.51 mm) ID x 30 feet (9.2 m), for flow rates less than or equal to 10ml/min.
760-0605	PEEK tubing, 1/16" (1.6 mm) OD x 0.030" (0.76 mm) ID x 30 feet (9.2 m), for flow rates greater than 10ml/min.
760-0650	DuoFlow F10 Tubing Kit. Includes premade tubing for basic installation of DuoFlow with F10 Workstation system.
760-0652	DuoFlow F40 Tubing Kit. Includes premade tubing for basic installation of DuoFlow with F40 Workstation system.
760-2002	Maximizer Tubing Kit. Includes premade tubing for buffer connection.
760-2046	DuoFlow pH Monitor Tubing Kit.

Fraction Collectors

741-0002	BioFrac Kit 110/120V. Includes the base unit, 100V power cord, Fittings kit, Diverter valve, Instruction manual, and Rack set F1 (2 x flatpack, 12-13 mm).
741-0003	BioFrac Kit 220/240V. Includes the base unit, Fittings kit, Diverter valve, Instruction manual, and Rack set F1 (2 x flatpack, 12-13 mm).
741-0008	BioFrac Diverter valve.
741-0010	BioFrac Rack set F1 (2 x flatpack, 10-13mm).
741-0011	BioFrac Rack set F2 (2 x flatpack, 15-16mm).
741-0012	BioFrac Rack set F3 (2 x flatpack, 18-20mm).
741-0013	BioFrac Rack set H1 (4 x flatpack, 1.5 ml microtubes).
741-0014	BioFrac Rack set H2 (4 x flatpack, 0.5 ml microtubes).
741-0015	BioFrac Rack set H3 (4 x flatpack, 16 mm scintillation vials).
741-0016	BioFrac Rack set H4 (4 x flatpack, 30 mm large scintillation vials).
741-0017	BioFrac Ice Bath & Microplate Rack.
741-0018	BioFrac Prep-20 Preparative Rack 741.

731-8238	Model 2128 Diverter Valve.
731-8122	Model 2110 Fraction Collector, 110 V.
731-8120	Model 2110 Fraction Collector, 220 V.
760-0410	Model 2110 Diverter Valve, SVT3-2.
731-8130	Model 2110 Carousel, 80-tube capacity.
731-8135	Model 2110 Micro Tube Adapter.
731-8136	Model 2110 Carousel Dust Cover.

Pumps

731-9001	Econo Gradient Pump, 100/120 V. Includes Tubing and Fittings kit.
731-9002	Econo Gradient Pump, 220/240 V. Includes Tubing and Fittings kit.
731-8140	Model EP-1 Econo Pump, 100/120 V.
731-8142	Model EP-1 Econo Pump, 220/240 V.

Chart Recorder

731-8250	Model 1327 Chart Recorder, with USA, Canada, Japan, Mexico, Taiwan, and Latin America power adapter.
731-8253	Model 1327 Chart Recorder, with UK, Commonwealth power adapter.
731-8254	Model 1327 Chart Recorder, with Australia, New Zealand power adapter.
731-8255	Model 1327 Chart Recorder, with European power adapter.

Cables

750-0650	System Cable 17 (instrument bus communication), 4 feet (1.2 m).
750-0651	System Cable 18 (instrument bus communication), 12 feet (3.7 m).
750-0652	System Cable 19 (instrument bus communication), 30 feet (9.2 m).
750-0655	System Cable 21 (instrument bus communication), 100 feet (30 m).
760-1309	System Cable 24, QuadTec analog to bare wire, includes two cables to connect to two SIMs.
760-1307	System Cable 25, QuadTec RS232, connects QuadTec to ICM.
760-1321	System Cable 26, ICM power cord, connects to DuoFlow Workstation.
760-2004	System Cable 30 (bus communication), 1 foot (30.5 cm).
760-2032	System Cable 31, USB Bitbus Communicator to controller.
731-8262	System Cable 2 (mini-DIN to Model 1327 DIN).
731-8264	System Cable 4 (mini-DIN to rec banana).
731-8265	System Cable 5 (Workstation AUX bare wires to DB-9).
731-8267	System Cable 7 (mini-DIN to bare wires).
750-0653	System Cable 20 (mini-DIN to DIN).

Miscellaneous

760-0135	Starter Kit.
720-0001	UNO Q1 Column.
760-0001	BioLogic DuoFlow Instruction Manual.

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