Miltenyi Biotec

autoMACS™ Pro Separator

User manual, version 2

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User manual

Version 2.0

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Thank you for choosing a Miltenyi Biotec product.

The autoMACS™ Pro Separator is an innovative instrument for automated multisample labeling and separation of various cells types. At the touch of a button, target cells are magnetically labeled, separated and eluted in a fully automated fashion.

Purify and progress

1 Important information

Please read before use!

Please read all information contained in this user manual before use. Failure to read and follow these guidelines could lead to improper or incorrect use, handling or care of your instrument and could cause hazards to users, unpredictable results, device malfunction or damage, premature wear and reduced life time of the instrument, and may void your warranty.

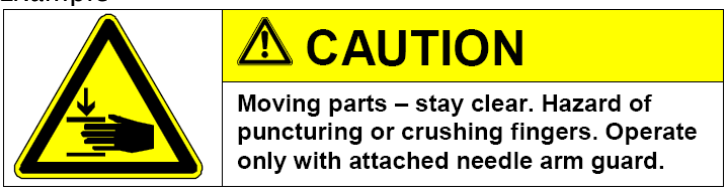
Keep this user manual in a safe place, accessible for anyone using the autoMACS Pro Separator.

This chapter describes the safety instructions and site requirements for your autoMACS Pro Separator. The following warnings and cautions are provided to help you prevent injury to yourself or damage to the device.

1.1 Symbols and hazard levels

Setup of safety notices

Example



The safety notices inform the user about potential risks if warnings and precautions outlined below are not followed. The icon on the left side specifies the risk. The hazard level at the top classifies the hazard, as mentioned above. The level, type, and source of the hazard as well as potential consequences, prohibitions, and measures are pointed as follows.

Symbols

The following chart is an illustrated glossary depicting the symbols that are used in this user manual and on the autoMACS Pro Separator.

⚠ CAUTION	Indicates a hazard situation, which if not avoided, could result in minor or moderate injury.
⚠ WARNING	Indicates a hazardous situation which, if not avoided, could result in death or serious injury.
	Attention, consult the User Manual for further instructions and proceed with caution.. Warnings include the risk of damage to the equipment, severe personal injury, or loss of life
	Hazard of crushing and shearing. Risk of crushing and shearing of bodily parts due to mechanical hazards.
	Laser radiation Risk of serious eye and skin injuries.
	Strong permanent magnet Contains a strong permanent magnet. Magnetic devices can interfere with electronic devices or damage magnetic information carriers.
	Risk of contamination if biohazardous material is used. Indicates the risk of loss of life, severe injury to the instrument operator, or equipment damage due to potentially dangerous biological material.
	Indicates the risk of loss of life or severe injury to the instrument operator due to hazardous voltage.
	Protective conductor terminal Symbol is attached on the inside of the instrument. Warning for service personnel.
	On (supply)
	Off (supply)

1.2 Warnings and precautions

The autoMACS Pro Separator employs state-of-the-art technology. It is a computer-controlled device for the automated separation of magnetically labeled cells using MACS Technology. The MACS MiniSampler connects to the autoMACS Pro Separator and thus represents a part of the cell separation device. The autoMACS Pro Separator and the MACS MiniSampler are designed to operate safely after installation and when used by trained personnel according to general safety practices and the instructions set forth in this user manual. The guidelines in this section explain the potential risks associated with the operation of the instrument and provide important safety information in order to minimize these risks. By carefully following the instructions, you can protect yourself and the equipment from potential hazards and create a safe work environment. If this instrument is used in a manner not specified by the manufacturer, protection may be impaired.

IMPORTANT: Please read and follow all operating instructions in this user manual and pay attention to all warnings displayed on the instrument. Retain this user manual and any other safety and operating instructions provided with the instrument in a place accessible to all users for future reference.

IMPORTANT: The autoMACS Pro Separator is intended for indoor use only. Do not use the instrument in areas classified as hazardous locations such as oxygen-laden environments.

Contact your local authority governing electrical power supply, building constructions, maintenance, or safety for more information regarding the installation of the equipment.

If you have a serious concern regarding the safe use of your instrument, please contact your authorized Miltenyi Biotec service provider or call Miltenyi Biotec Customer Service.

1.3 General precautions

To reduce potential risks associated with operating the autoMACS Pro Separator, please observe the following general precautions. Failure to observe these precautions could result in fire, bodily harm, and/or damage to the instrument.

1.3.1 Hazard of electric shock and spread of fire

⚠ WARNING

Hazardous voltages. Risk of loss of life or severe personal injury. Unplug before cleaning. Do not use
- if device is opened or damaged,
- if liquids have been spilled into device,
- if objects entered device through ventilation slots.

Warning: Electrical devices pose the risk of an electric shock. To reduce the risk of an electric shock, do not open any cover other than the front access covers of the autoMACS Pro Separator nor any other accessory hardware supplied by Miltenyi Biotec. All other covers of the device and accessory hardware are to be removed by authorized personnel only. Special care must be taken while handling fluids. Clean up spillages immediately. Do not allow fluids to enter the interior of the device. Unplug the power cord before manually cleaning the autoMACS Pro Separator.

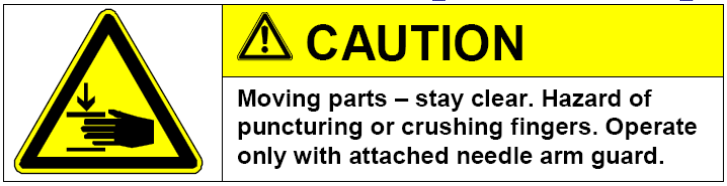
Warning: A potential risk exists if an opened, dropped or damaged autoMACS Pro Separator is used, if liquids are spilled into the instrument, if an object has entered the instrument through the ventilation slots, or if an object has been dropped into the instrument. If flames or smoke appear immediately switch off the the autoMACS Pro Separator, unplug the instrument from the electrical outlet, and contact an authorized Miltenyi Biotec service provider or the Miltenyi Biotec Customer Support team. Use of a damaged instrument or an instrument with a damaged power cable is expressly prohibited.

1.3.2 Strong magnetic field

Warning: The autoMACS Pro Separator is equipped with an extremely powerful magnet. Keep any magnetic information carriers (such as credit cards, magnetic tapes and floppy disks) and any electronic equipment (such as hearing aids, pacemakers, measuring and control instruments, computers, and watches) at a distance of at least 20 cm from the magnet cover. These items may be affected or damaged by the magnetic field.

Figure 1.1 Location of warning sign for strong permanent magnet.

1.3.3 Hazard of crushing and shearing



CAUTION: Do not open the front access covers while the device is in operation. Do not obstruct the movement of the automated arm and accessory hardware during operation. Keep fingers etc. away from all moving parts of the autoMACS Pro Separator and accessory hardware, to avoid crushing or shearing injuries, or damage to the device. Do not touch fluid pumps or adjust the tubing, while the device is in operation. Always switch off the device before adjusting any part of the fluidic system. Always stop or abort a procedure before handling accessory hardware, e.g. MACS MiniSampler, or loading/removing tubes from the tube rack placed on the sampler. Do not circumvent any safety measures or devices.

Figure 1.2 Open circle shows warning sign for 'hazard of crushing and shearing'.

1.3.4 Laser radiation

Warning: The device is equipped with four vertical cavity surface emitting lasers (VSCLs) for automated rack detection (Class 1M). The radiation is not visible. Do not view directly with optical instruments (e.g. lenses, magnifying glasses, and microscopes). Viewing the VSCL port within 100 mm distance with optical instruments could be hazardous to the eye.

The device is also equipped with a 2D Code Reader which uses a visible semiconductor laser as a target pointer for adjusting the reading position and powerful light emitting diodes (LEDs) for illuminating the reading area.

Do not look directly at laser or LED radiation or reflected laser or LED radiation from a mirrored surface. Otherwise, eye injury may result. Do not intentionally direct the laser beam at others.

Do not disassemble, modify or remove the installed laser or LED radiation sources or their mounting rackets. The laser or LED radiation sources do not automatically stop emitting when disassembled.

Radiation of disassembled units may lead to eye injuries.

Be careful of the path of the laser beam or reflection from a mirrored surface. Take care during installation of the autoMACS Pro Separator that the path of the laser beam is not at the same height as that of the human eye during operation.

Do not allow water, oil, dust, or other foreign substances to stick to 2D Code Reader aperture window. This may cause read errors. Be sure to stop the laser emission before cleaning the scanner. Otherwise, exposure to the laser may cause eye injury. Use a soft, dry cloth to wipe any substances from the scanner. Do not use alcohol or other cleaning substance.

The autoMACS Pro Separator is classified as a Class 1M laser product per standard IEC 60825-1: 1993 + A1: 1997 + A2: 2001.

CAUTION: Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.

Figure 1.3 Position of lasers. Invisible rack detection lasers are located within the rectangle area. The 2D code reader (visible) is located within the open circle.

1.4 Secure installation

This section describes the requirements your site must meet for safe installation and operation of your autoMACS Pro Separator. Read the instructions in this section and ensure that your site is properly prepared before you connect the instrument to its power source.

When planning your site layout and equipment locations, keep in mind the precautions described in this section to help avoid instrument failures and reduce the possibility of environmentally caused shutdowns.

IMPORTANT: At all times, local working area safety instructions, laboratory policies, and standards regarding laboratory health and safety and prevention of accidents must be adhered to.

1.4.1 Mounting accessories

Do not place the autoMACS Pro Separator on an unstable table, cart, stand, tripod, or bracket. As a consequence, the instrument might fall down. This may cause serious bodily harm and/or serious damage to the instrument. Use only on a table, cart, stand, tripod, or bracket recommended by Miltenyi Biotec or sold with the instrument. Do not place the autoMACS Pro Separator within a built-in apparatus or a confined space such as a shelf rack unless the apparatus has been specifically designed to accommodate the instrument, proper ventilation is provided, and the mounting instructions for the instrument have been followed.

1.4.2 Air circulation

Ambient air temperature might not be adequate to cool the autoMACS Pro Separator to acceptable operating temperatures without adequate circulation. Make sure that the room in which you operate the instrument has adequate air circulation. The instrument should not be placed next to radiators, heat registers, stoves, or other pieces of equipment (including amplifiers) that produce heat. Allow sufficient air circulation around the autoMACS Pro Separator—at least 15 cm on all sides—during operation to ensure adequate cooling of the instrument. Prevent direct exposure of the instrument to sunlight. Slots and openings of the instrument are provided for ventilation and should never be blocked or covered, as these ensure reliable operation of the autoMACS Pro Separator and protect the device from overheating. Never push a foreign object through an opening into the instrument.

1.4.3 Water and moisture

Do not use the instrument in a wet or damp location. Avoid high humidity or condensation and protect the machine against water splashes.

1.4.4 Grounded (earthed) product

The instrument is equipped with a three-wire electrical grounding-type plug that has a third pin for grounding. This plug only fits into a grounded power outlet. This is a safety feature. Do not try to insert the plug into a non-grounded power outlet. If you cannot insert the plug into the outlet, contact your local electrician to replace the outlet.

1.4.5 Power sources

The instrument should only be operated from a power source indicated on the product's electrical ratings label. If you have questions about the type of power source to use, contact your authorized Miltenyi Biotec service provider or local power company. Do not use extension cords or power strips. Do not overload an electrical outlet. The overall system load must not exceed 80% of the branch circuit rating.

1.4.6 Accessibility

Make sure that the main switch as well as the connector for the power cable are easily accessible and located as close to the operator of the instrument as possible. If it is necessary to disconnect the power supply, unplug the cable from the power outlet.

1.4.7 Peripheral devices

Only peripheral devices that comply to UL 60950 are allowed to be connected to the RS232 connector labeled "COM". The connector labeled "RS232/AUX" is not in use. In addition, only original autoMACS Pro Equipment should be attached to the connectors labeled "External CAN", "CAN1", and "CAN2". The voltage levels on these connectors shall not exceed hazardous voltage levels of 30 V rms. and 42.4 V peak or 60 Vdc. Only the autoMACS Pro Bottle Sensor Cable should be attached to the "Bottle Sensor" connector. Only a 2D code reader recommended by Miltenyi Biotec should be connected to the "RS232/BCR" connector. External laser devices connected to the connector labeled "RS232/BCR" have to comply with the standard IEC 60825-1. Only use connector cables less than 3 m in length.

1.5 Secure operation, maintenance, transport and disposal

Observe the following instructions to ensure secure operation, maintenance, transport, and disposal of your autoMACS Pro Separator.

IMPORTANT: At all times, local working area safety instructions, laboratory policies, and standards regarding laboratory health and safety and prevention of accidents must be adhered to.

1.5.1 Secure operation

If the instrument is not working properly and instructions or messages on the display screen advise to contact technical service, secure operation is no longer possible. Immediately switch off the autoMACS Pro Separator, unplug the instrument from the electrical outlet, and contact an authorized Miltenyi Biotec service provider or the Miltenyi Biotec Customer Support team.

1.5.2 Servicing

IMPORTANT: Unless otherwise specifically noted in this User Manual or other Miltenyi Biotec documentation, do not service the autoMACS Pro Separator yourself. Servicing and repair must be performed by qualified service personnel. Improper or incorrect servicing or repair of your autoMACS Pro Separator can cause hazards to users, lead to unpredictable results, device malfunction or damage, premature wear and reduced life time of the instrument, and may void your warranty.

Inquire with your local Miltenyi Biotec representative about Miltenyi Biotec's extensive instrument service and support arrangements, or see www.miltenyibiotec.com/support.

IMPORTANT: When replacement or spare parts are required, make sure that the service provider uses only genuine Miltenyi Biotec parts or third-party parts specified and recommended by Miltenyi Biotec. Using unauthorized replacement or spare parts can cause malfunction of the device and impair cell separation results. Miltenyi Biotec does not honor any warranty or accept any responsibility for device failure or damages resulting from the use of inappropriate replacement or spare parts. After completing any service or repair work, have your authorized Miltenyi Biotec service provider perform all safety checks required by the repair procedure to ensure that the instrument is in proper operational condition.

Only use options and upgrades recommended by Miltenyi Biotec.

1.5.3 Cleaning

Unplug the autoMACS Pro Separator from the outlet before cleaning. Do not use liquid or aerosol cleaning agents; always use a damp cloth.

1.5.4 Hazardous material

⚠ WARNING

Risk of loss of life if biohazardous material is used. Wear protective gloves, protective clothing, and safety glasses to prevent contact with skin and eyes. Operate device in a safety hood. Decontaminate device after spilling of biohazardous material.

If biohazardous material is or has been used, the operator shall choose and wear personal safety equipment in accordance with warnings and precautions for the used substances. Wear protective gloves, protective clothing, and safety glasses to prevent contact with skin and eyes. Also protect mouth and nose as aerosols might leak from the system (e.g. Washing Station). Defective or inadequate safety equipment might endanger the operator. The autoMACS Pro separator shall be operated in a safety hood if hazardous or unknown materials are processed. If hazardous material has been used or spilled, care must been taken to thoroughly decontaminate the system. For details, see section 7.15.

Always inspect the fluidics system (complete tubing set, bottles and their closures, valves, columns, diluter and needles) before switching on the device. If leakage has been detected, replace all damaged parts before switching on the device. If damaged parts cannot be replaced, unplug and do not use the device. Failure of parts containing biohazardous material or liquids that have been in contact with such material could cause a hazard.

Columns, tubes, and any other consumables that were in contact with biohazardous samples shall be autoclaved prior to disposal. Liquid waste shall be autoclaved or decontaminated using a disinfectant that is appropriate for the specific pathogen, e.g. 10% bleach, isopropyl alcohol, or 70% ethanol.

Waste disposal must be in accordance with any local regulations.

Figure 1.4 Warning signs for biohazard located on lower facing panel of the autoMACS Pro Separator (left) and top of autoMACS Pro Separator fluid bottle (right).

Flammable
70% ethanol is used in Sleep and Store programs. The solvent is flammable. Therefore, keep the instrument away from fire.

1.5.5 Transport

The autoMACS Pro Separator should be transported with care in packaging specified by Miltenyi Biotec. Internal damage can occur, if it is subjected to excessive vibration or if it is dropped. If the instrument needs to be shipped back to the manufacturer for service, decontaminate the instrument from any hazardous material prior to shipment. If you have questions regarding proper decontamination or shipment, please contact technical service for assistance. See section 7.15 for further information on instrument decontamination.

1.5.6 Instrument disposal

Please contact technical service for assistance if you wish to dispose of your instrument.

1.5.7 Electromagnetic compatibility

Changes or modifications of the equipment unless expressly approved by Miltenyi Biotec may void your authority to operate the equipment pursuant to 47 CFR §15.

NOTE: This equipment has been tested and found to comply with the limits for a Class B digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a residential installation. This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- Reorient or relocate the receiving antenna.
- Increase the separation between the equipment and receiver.
- Connect the equipment into an outlet on a circuit different from that to which the receiver is connected.
- Consult the dealer or an experienced radio/TV technician for help.

2 Introduction

2.1 MACS® Technology

MACS Technology has become the standard method in cell separation. It is based on the use of MACS MicroBeads, MACS Columns, and MACS Separators—strong permanent magnets. In a first step, surface antigens are magnetically labeled in a highly specific manner with monoclonal antibodies coupled to MACS MicroBeads. After magnetic labeling, the cells are passed over a MACS Column placed in a MACS Separator. Non-labeled cells flow through and can be collected; labeled cells are retained in the column and can be released after removing the column from the magnet. Thus, both labeled and non-labeled cell fractions can efficiently be isolated with MACS Technology. The entire procedure is fast, easy to handle, and gentle to cells, leading to the purification of viable and functionally active cells that can immediately be used for further experiments.

2.2 Super-paramagnetic MACS® MicroBeads

MACS MicroBeads are super-paramagnetic particles of approximately 50 nanometers in diameter, being comparable to the size of a virus. MicroBeads do not change the scatter properties of the cell in the flow cytometer or influence the light-microscopic appearance of the cell. They form a stable colloidal suspension and do not precipitate or aggregate in magnetic fields. MACS MicroBeads are composed of a biodegradable matrix made of iron oxide and polysaccharide. Hence, it is not necessary to detach cell-bound beads after the separation process, saving hands-on time. Usually, MACS MicroBeads do not alter structure, function, or activity status of labeled cells, and they are not known to interfere with subsequent experiments. The isolated cells can be used directly for subsequent studies or cell culture.

MACS Cell Separation Reagents are highly specific. Thus, it is possible to label, for example, B cells and T cells as well as rare target cells with frequencies as low as 10^{-8}. MACS Technology allows to isolate viable antigen-specific T cells, $CD133^+$ hematopoietic stem and progenitor cells, subpopulations of $CD34^+$ hematopoietic progenitor cells, and disseminated carcinoma cells circulating in the peripheral blood. Finally, MACS MicroBeads offer an extremely flexible tool to isolate many cell types from many species. Several hundreds of reagents for the isolation of human, mouse, rat, and non-human primate cells as well as reagents for indirect labeling of many other cell types are available.

2.3 A column-based, high-gradient magnetic cell separation

The MACS Separation Unit consists of a powerful permanent magnet. If a MACS Column is placed into the magnetic field of the Separation Unit, the small ferromagnetic structures of the column matrix disturb this homogenous magnetic field and, thereby, produce high magnetic gradients. In their immediate neighborhood, the ferromagnetic structures generate magnetic forces 10,000-fold greater than in conventional geometries, allowing to retain target cells labeled with minimal amounts of MACS MicroBeads. Additionally, the columns are rapidly demagnetized when the column is removed from the separator, making the recovery of the retained cells easy and gentle. This method allows separation of viable cells from samples containing a few hundred cells up to 10^{11} total cells. Within approximately 15 minutes, the cells are labeled with MACS MicroBeads—nano-sized superparamagnetic particles coupled to specific antibodies. The actual separation of cells over the column is completed within minutes.

2.4 The autoMACS™ Pro Separator

The autoMACS™ Pro Separator is a benchtop magnetic cell sorter that allows gentle sorting of more than 10 million cells per second from a sample of up to 4×10^{9} total cells. The instrument is designed for use with any MACS Cell Separation Reagent for research applications. Thus, it is possible to choose between different cell separation strategies according to the respective experimental design—from positive selection of abundant or rare cells to the isolation of untouched cells by depletion of non-target cells. 12 preset separation programs simplify and standardize the application.

IMPORTANT: The autoMACS Pro Separator is intended for research applications only and not for diagnostic or therapeutic use.

The autoMACS Pro Separator features automated sample labeling (autolabeling), sample loading, elution of the negative, non-labeled as well as the positive, labeled cell fractions. Up to six samples can be processed in one programming step. Furthermore, automated procedures for maintenance of the system are included. Different wash programs are available to rinse the columns before a new separation is performed. One pair of columns can be used for up to 100 cell separations or for 2 weeks, whichever comes first. The TFT color touchscreen with intuitive screen menus makes operation and monitoring of the instrument intuitive and easy. Finally, standard MACS Fluid Containers, which are directly attached to the instrument, and ready-to-use sterile MACS Buffers are available for maximum convenience.

The autoMACS Pro Separator is supplied with the MACS MiniSampler and therefore offers an additional sampling option. This new feature allows the sequential processing of multiple samples without further manual handling. The sampler is

supplied with three different tube racks and an additional reagent rack. The sampler and tube racks are automatically detected by the autoMACS Pro Separator adding to its operational efficiency.

The user manual aims at explaining the principles of cell sorting with the autoMACS Pro Separator and to assist you by providing step-by-step protocols for cell labeling, separation, and quality control. Tips and hints in the troubleshooting section, as well as general protocols, and special separation protocols are intended to help optimize your magnetic cell separations.

2.4.1 Description of the autoMACS™ Pro Separator

The autoMACS Pro Separator has been specifically designed for the automated processing of multiple samples. At the touch of a button, target cells are magnetically labeled, separated and eluted in a fully automated fashion.

Figure 2.1 Front image of the autoMACS Pro Separator – the access cover and bottom cover were made transparent for the purpose of illustration.

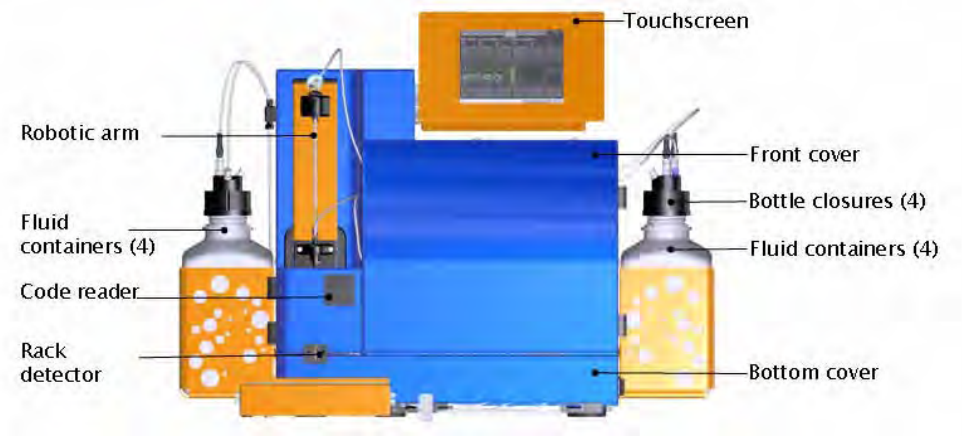


Figure 2.2 Front view of autoMACS Pro Separator

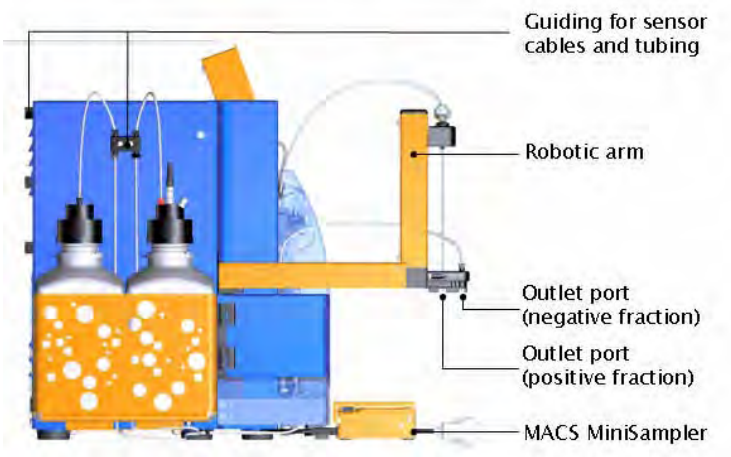


Figure 2.3 Left side view of the autoMACS Pro Separator

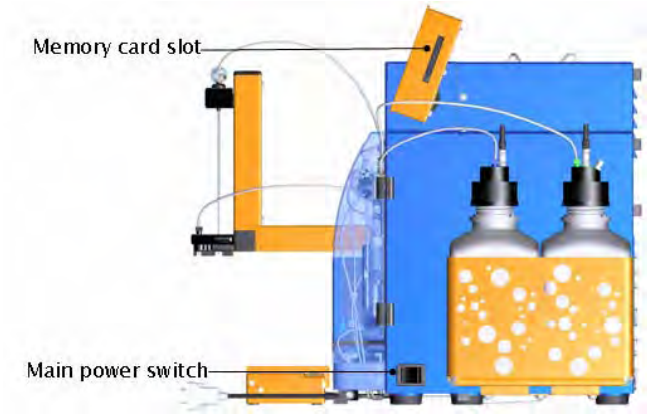


Figure 2.4 Right side of the autoMACS Pro Separator

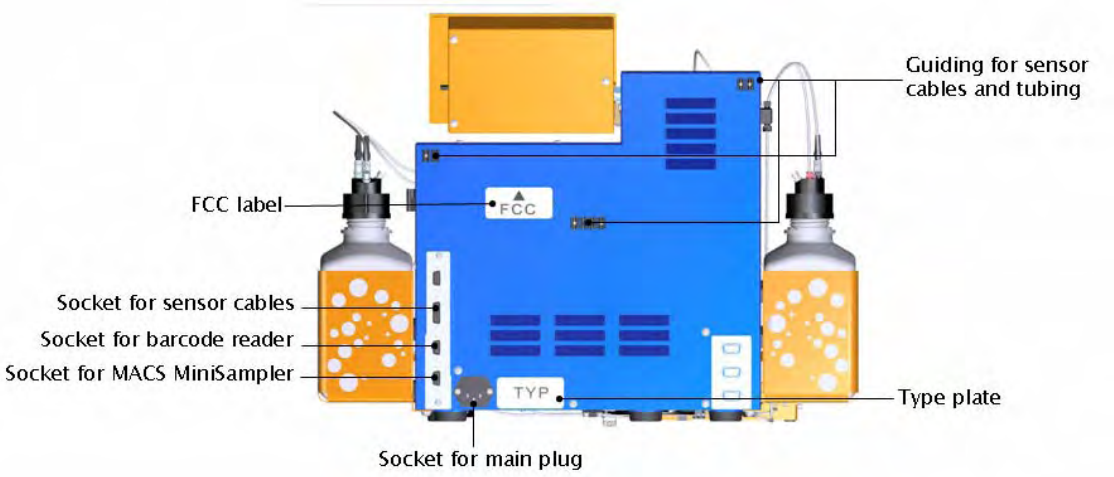


Figure 2.5 Rear view of the autoMACS Pro Separator

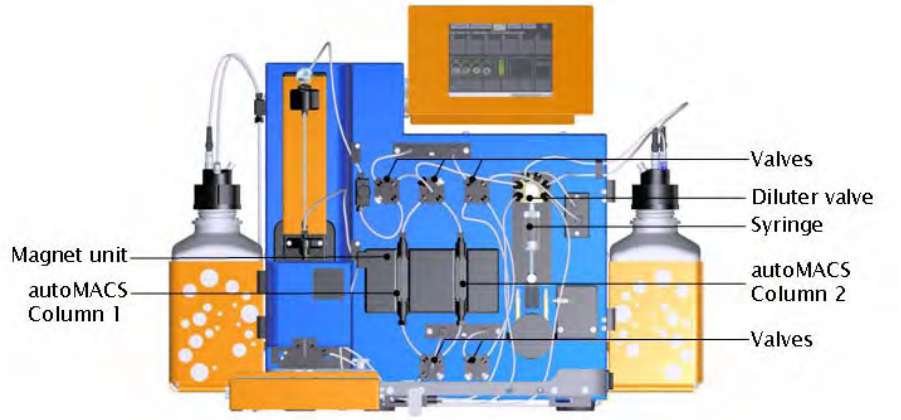


Figure 2.6 Front of autoMACS Pro Separator with access and bottom covers removed

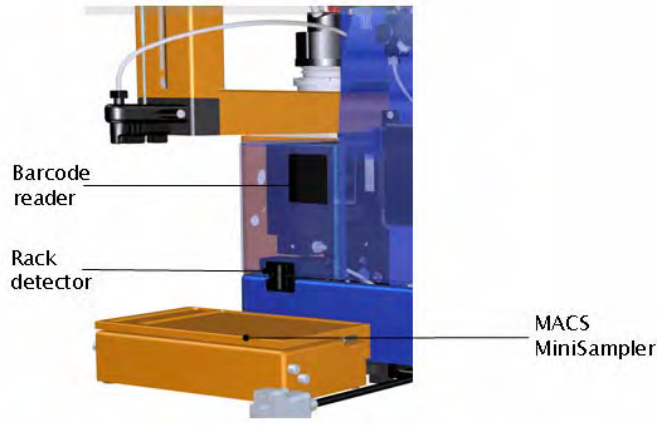


Figure 2.7 Expanded view of the autoMACS Pro Separator 2D code reader (“barcode reader”)

Integrated computer for control of cell processing

All interactions with the computer are performed with a TFT color touchscreen (Figure 2.2). A memory card is used to run all programs and to log processes.

Automated arm with ports for sample uptake and release of cell fractions

The automated arm (Figure 2.3) is a computer-controlled part of the autoMACS Pro Separator. It holds two ports, one for sample uptake and release of the magnetically labeled, positive cell fraction and one for release of the non-labeled negative cell fraction. The automated arm moves in y and z directions. The ports are automatically washed in the autoMACS Pro Washing Station during and after the separation process to prevent cross-contamination between samples.

Access covers

The front door (Figure 2.2) is opened sideways to allow access to the parts of the fluidic system that require periodic maintenance by the user. This includes the autoMACS Columns, pump syringe, and upper valves. The bottom cover (Figure 2.2) gives access to the lower valves and can be removed by pulling gently. The washing station cover (Figure 2.2) is opened sidewards giving access to the washing station, the peristaltic pump, and the tubing of the autoMACS Pro Washing Station.

Fluid containers and fluid container baskets

Two baskets (Figure 2.2) holding two fluid containers each, are located at each side of the instrument. Fluid containers are connected to the autoMACS Pro System with color-coded tubing and sensors at the bottle closures for fluid level control (Figure 2.2).

MACS MiniSampler, Chill Racks and MACS Reagent Rack

Figure 2.8 Rear view of MACS MiniSampler with MACS Reagent Rack and Chill Rack 5

The MACS MiniSampler (Figure 2.8) can be loaded with one of three different cooling tube racks that carry cell samples and fraction collection tubes and the MACS Reagent Rack 4. The upper plate of the MiniSampler moves in an x-direction and aligns the tube openings with the port of the automated arm. The guiding of the MiniSampler is directly attached to the corresponding slot below the washing station. When attaching the MiniSampler sensor cable to the corresponding socket at the rear of the instrument

the MiniSampler will be automatically detected. The type of tube rack carried by the MiniSampler is automatically recognized by the rack detector after starting the separation process. During operation, the tube rack should be covered with the MiniSampler lid that is connected to the lid guiding (Figure 2.8). The MiniSampler can be disconnected from the autoMACS Pro Separator by pulling is up on the front side and pulling it towards the user.

Plugs, connections and guidings

Sockets for the main plug (Figure 2.5), the fluid sensor cables, the 2D code reader cable and the MACS MiniSampler are installed at the rear of the instrument. Additional sockets are implemented for further instrument development. The main power switch is located at the right hand side of the instrument (Figure 2.5). Several guidings at the rear and sides of the instrument ease the safe connection of tubings and sensor cables.

2D code reader ("barcode reader")

The 2D code reader is used to read 2Dimensional codes. Each MACS Reagent is uniquely identified by a 2D code label. Vials scanned using the 2D code reader are automatically recognized by the software, which also uploads corresponding protocol information. For information regarding installation of the 2D code reader please refer to section 3.2.11.

The cell separation unit

The central part of the cell processing unit consists of a magnet (Figure 2.6) and two autoMACS Columns (Figure 2.6). Once installed, the autoMACS Columns become part of a closed fluidic system and can be used for up to 14 days OR 100 separations, whichever comes first. Fluids are put through the fluidic system with the help of a syringe pump and five valves.

2.4.2 2D code reader

The autoMACS Pro Separator is equipped with a 2D code reader that uses lasers and powerful light-emitting diodes (LEDs) for illuminating the reading area. The 2D code reader light is classified as a Class 1 laser product per standard IEC 60825-1:1993 + A1:19976 + A2: 2001 (maximum output 116 µW; wavelengths 655 nm, pulse duration 1 ms). Please refer also to section 1.3.4 (Important Information) of the autoMACS Pro Separator Manual for associated warning and precautionary information. Refer to section 3.2.11 for information on installation of the 2D code reader.

2.4.3 autoMACS™ Pro Fluid Containers

Three containers hold the fluids required for operation of the autoMACS Pro Separator. An additional container holds the waste fluid. The bottle closures, the fluid sensor cables, and the tubing connectors are color-coded. The specific position for each container is indicated by individual symbols on the autoMACS Pro Separator. See section 7.15 for information on disposal of biohazards.

2.4.4 Capacity

The autoMACS Pro Separator uptake volume ranges from 0.2 mL to 50 mL with standard programs. The uptake volume range for whole blood ranges from 0.25 – 15 mL. Depending on the labeling strategy, whole autoMACS Columns can retain up to 2×10^8 labeled cells from samples containing from 10^5 up to 4×10^9 total cells. The mixing volumes for use of the autolabeling feature are as follows:

- From 0.2 mL to 2.5 mL (Chill Rack 5); 0.2 mL to 6.5 mL (Chill Rack 15).
- From 4 mL to 8.4 mL (Chill Rack 50).

3 Assembly and installation

3.1 Unpacking the autoMACS™ Pro Separator

Read through the following instructions carefully before commencing the installation procedure. Before opening the transportation box, check for any visible external damage to the box. Check also to see if the shock and position indicators (if present) suggest incorrect transportation of the instrument. If there is apparent damage please contact technical support for assistance (see section 12).

1) Open the box and remove the top layer of the packaging to reveal the instrument and associated packaging.

> **Note:** The top layer holds the autoMACS Pro user manual, the short instructions, and various bags containing accessories. Carefully remove these parts.

2) Remove boxes containing the MACS MiniSampler and cover, and the MACS Cooling Tube Racks.

Figure 3.1 Packing format of the autoMACS Pro Separator and accessories.

3) Remove the foam packaging from both sides of the autoMACS Pro Separator.

> **Note:** Two persons are required to lift the autoMACS Pro Separator. The instrument must be gripped at the base of the orange bottle baskets located at both sides of the device. Note that the instrument is heavier at the front. Ensure the front of the instrument is stabilized while lifting it.
>
> Due care must be taken while lifting the autoMACS Pro Separator. Miltenyi Biotec accepts no liability for potential injuries sustained during lifting and/or movement of the device.

4) Place the instrument onto a stable worktop surface, e.g., laboratory bench. Remove the plastic bag surrounding the device.

5) Carefully remove the uptake port needle from the foam packaging.

Figure 3.2 Close-up of the autoMACS Pro Separator packaging. The needle arm and underside of the touch screen are supported by foam.

6) Place the uptake port needle into its guiding at the needle arm.

Figure 3.3 The needle port is positioned as illustrated above.

Note: Ensure that the tubing connected to the uptake port needle can move freely when the needle arm extends, or when the needle moves into the sample uptake position.

3.2 Installation of the autoMACS™ Pro Separator

Note: Before installation carefully read the chapter Important information (section 1).

The autoMACS Pro Separator is a benchtop instrument that fits in laminar flow hoods or safety cabinets. If the instrument is placed in a laminar flow hood, the following accessories might be required: autoMACS Pro Laminar Hood Plate (# 130-093-246) and, optionally, autoMACS Pro Angle Connector Kit (# 130-093-245). The Laminar Hood Plate provides a stable surface, even on potentially bending surfaces. The angle connectors reduce the depth of the instrument to 455 mm (including MACS MiniSampler) for placement in a location with limited space.

The operating environment should be stable and vibration-free, dust-free, sufficiently ventilated, and free from sources of electromagnetic radiation.

Note: Before operating the autoMACS Pro Separator for the first time, carefully read the user manual and contact your local Miltenyi Biotec representative for assistance.

3.2.1 Connecting the power cord

1) Note the position of the power socket on the rear panel of the autoMACS Pro Separator (Figure 2.5).
2) Ensure that the main power switch is in position "0" before connecting the power cord.

3.2.2 Connection of fluid containers and fluid sensor cables

Operating the autoMACS Pro Separator requires Running Buffer, Washing Solution, and Storage Solution (70% ethanol). It is recommended to operate the instrument with ready-to-use MACS Buffers. The autoMACS Pro Separator is delivered with four empty fluid containers which are connected to the instrument with specifically designed bottle closures. The bottle closures consist of a fluid uptake port or a fluid outlet port (waste container) as well as a sensor for measuring electrolyte conductivity. The fluid containers, bottle closures, and fluid sensor cables are color-coded for easy handling (see table below).

Container	Symbol	Container	Symbol
Running Buffer (blue)		Storage solution (black)	
Washing solution (green)		Waste (red)	

Table 3.1 Symbols and color coding of fluid containers

Note: The 70% ethanol does not contain electrolytes. Therefore, the filling status of the ethanol container cannot be determined.

1) Install one fluid at a time. Please note the corresponding color coding (see Table 3.1 Symbols and color coding of fluid.
2) Unscrew bottle closures counter-clockwise and remove the empty container. Do not disconnect the color-coded tubing.
3) Do not open a fresh bottle until it is placed in the basket! Place the bottle in its appropriate position, remove the cap, and fasten the bottle closure.
4) Remove the packaging from the fluid sensor cables.
5) Note the color coding and connect each sensor cable to the respective bottle closure.

6) Attach the sensor cables to the cable guide at the back of the autoMACS Pro Separator.

7) Attach the sensor cable plug to the socket for sensor cables at the back of the autoMACS Pro Separator labeled “Bottle Sensor“ and fasten securely.

8) Connect the hydrophobic air filters (0.2 µm) to the appropriate connectors on the bottle closures.

Note: The correct positioning of each solution container—recognizable by the color code and the symbols—is crucial in order to perform successful procedures using the autoMACS Pro Separator.

To keep buffers sterile, each bottle closure should be equipped with a hydrophobic air filter. Avoid any contact of hydrophobic air filters with fluids as this may cause clogging of the filter.

When working with biohazardous samples, it is recommended to fill the waste container with 100 mL of disinfectant before use (e.g. MACS Bleach solution; order number #130-093-663). For proper disposal, please follow local regulations and carefully read the chapter Important information.

3.2.3 Installation of the MACS® MiniSampler and tube racks and reagent rack

The autoMACS Pro Separator is delivered with the MACS MiniSampler, three different tube racks and a reagent rack (MACS Reagent Rack 4). Once installed, the MiniSampler is automatically recognized by the autoMACS Pro Separator. Each tube rack has a barcode on the rear side that is detected upon starting the separation process.

1) Remove the transparent protection foil from the lens of the rack detection.

2) Note the positions of the MACS MiniSampler guiding (2) and its corresponding slot (1) located at the front of the instrument.

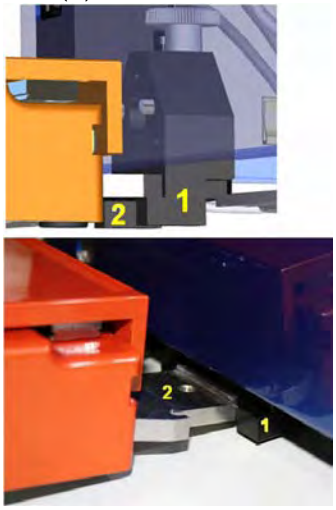


Figure 3.4 Location of the MiniSampler guide (2) and receiving slot (1) for the MACS MiniSampler.

3) Tilt the MiniSampler and slide the guiding into the receiving slot until resistance is met; lower the rack to a horizontal position i.e., the rack is locked in the position illustrated by the above figure.

4) Ensure that the MiniSampler is completely inserted and secure the connection by fastening the MiniSampler screw (3) as shown in the figure below.

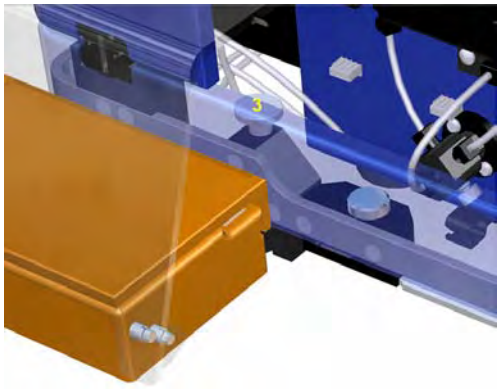


Figure 3.5 Securing the MiniSampler by fastening the MiniSampler screw.

5) Note the position of the lid guiding at both sides of the MiniSampler and attach the lid.

6) Place the MiniSampler cable underneath the autoMACS Pro Separator and connect it to the socket (4) labeled “External CAN “ at the rear panel of the instrument.

Figure 3.6 The MACS MiniSampler cable is attacted to socket 4 at the back of the instrument.

3.2.4 Positioning of cooling tube racks and the MACS Reagent Rack 4

1) Open the lid of the MACS MiniSampler.

2) Secure the MACS Reagent Rack 4 unto the MiniSampler into the left recess. The engagement hook has to snap into the undercut.

3) Set a cool tube rack (e.g. Chill Rack 15) unto the MiniSampler into the right recess ensuring that the rack barcode is facing the autoMACS Pro Separator.

Figure 3.7 Positioning the Chill Rack 15 adjacent to a MACS Reagent Rack 4 on the MACS MiniSampler.

Note: Racks can be pre-cooled for 3-4 hours at 2-8 °C. Do not cool below 0 °C since samples may freeze. If recognition of the tube rack fails, the instrument will display a screen for manual selection of the tube rack. Before confirming the choice, ensure that the rack is placed correctly into the recess.

3.2.5 Switching ON/OFF the autoMACS™ Pro Separator

The main power switch is located on the right side of the instrument in front of the container baskets ("I" indicates "On", "O" indicates "Off"). Switch on the autoMACS Pro Separator.

Figure 3.8 Location of the on/off switch

3.2.6 Installation of new software and instrument calibration

After installing an autoMACS Pro software update it is necessary to calibrate the instrument as instructed below. The autoMACS Pro Separator contains various control boards. During initialization of the instrument with a software card, all of these components are automatically checked for the currently installed software.

Note: Do not remove or insert a memory card while the instrument is turned ON. The memory card must remain in the unit. Removing the memory card during operation will abort all running processes.

Exchanging the software cards

1) Ensure the instrument is switched-off before proceeding.
2) Note the memory card slot at the right hand side of the touch screen.

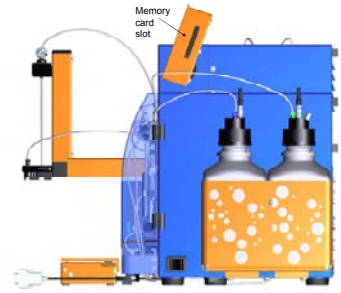


Figure 3.9 Location of the memory card slot.

3) Remove the old memory card by pressing the black release button.
4) Insert the new memory card.
5) Switch the instrument on. A dialog box will appear prompting the operator to proceed if desired.
6) Select **Update** to confirm that you wish to proceed.

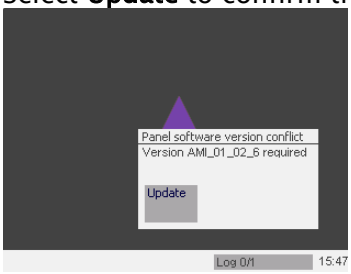


Figure 3.10 The user must confirm any update. The current software (panel software version) will be overwritten.

> **Note:** During the panel software update process, the display may turn black or white or may freeze or flicker. This process may take several minutes. The progress will be indicated by an acoustic signal.
> * If warning "7030" is reported confirm by selecting **OK**.
> * If warning "7016" is reported select **Ignore**.

3.2.7 Calibration of the autoMACS™ Pro Separator

The autoMACS Pro Separator is calibrated by using the two programs, **Calibr_1** and **Calibr_2**. Calibration is always necessary after installation of new software. Calibration 2 is always necessary when the pump syringe, the Diluter Valve or the tubing are changed.

Calibr_1 is used to calibrate the settings of the needle arm, i.e. alignment of the needle arm with the washing station, the MACS Cooling Tube Racks, and the bottom detection sensor control (x, y and z-axis).

Calibr_2 automatically calibrates the liquid volume control. This is crucial for the correct measurement and processing of the sample volumes. The fluidic system must

be filled with buffer before commencing this step, i.e., the **Rinse** program must be performed.

Calibration 1: Beginning the calibration sequence

1) Select menu **Option**
2) Highlight **User settings** and select **Calibr_1**
3) Select **Run**

> **Note:** If warning '7016' appears, select **Ignore**.

4) Select **Calibrate** to proceed. The first of five (1/5) calibration steps will begin, namely, calibration of the washing station.

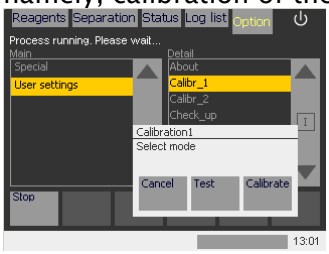


Figure 3.11 Performing calibration of the needle-arm: a 5-step calibration process

Calibration 1: Calibration of the washing station – step 1/5

1) Select **Use** to proceed with calibration. The needle arm will automatically move towards the washing station and should be located directly above the center of the rear opening of the washing station.

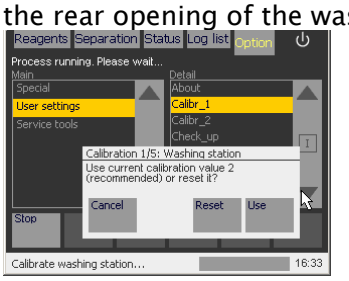


Figure 3.12 Calibration of the washing station. To use already saved settings select 'Use'. To reset calibration settings (not recommended) select 'Reset'.

2) Check the central positioning of the uptake needle by using the **Height** buttons Adjust by using use the **Move back** and **Move fwd** buttons.
Select **Done** after making necessary adjustments.

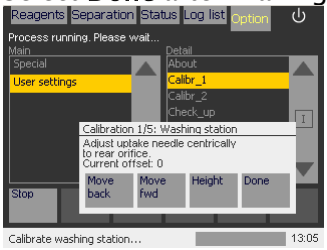


Figure 3.13 Having adjusted the position of the uptake needle, select 'Done' to finish calibration.

3) Select **Save** to save the new configuration.

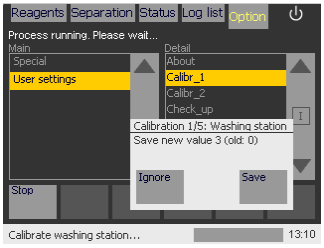


Figure 3.14 Saving new settings for calibration of the washing station.

Calibration 1: Calibration of the tube rack – step 2/5

1) Select **Calibrate** to continue with the calibration process.

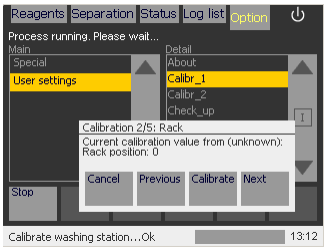


Figure 3.15 Calibration of the MACS Cooling Tube Racks

2) Place a MACS Cooling Tube Rack with sample tubes onto the MiniSampler. It is recommended to use a Chill 5 Tube Rack.

3) Select **Done** and then Select **Use** (recommended) to start the calibration using current settings.

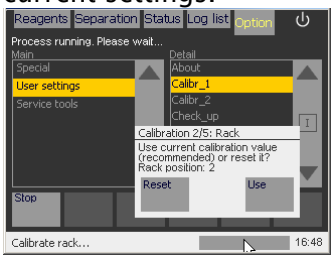


Figure 3.16 Select 'Use' to apply current settings for calibration of MACS Cooling Tube Racks.

4) Check the central positioning of the uptake needle on the bottom of the tube by using the needle navigation buttons (**Move back**, **Move fwd**, **Height**). Ensure the needle is positioned at the bottom center of the tube in row A of the tube rack. Select **Done** to continue.

5) Select **Save** to store the new settings.

Calibration 1: Calibration of the bottom detection – step 3/5

1) Select **Calibrate** to proceed with calibration.

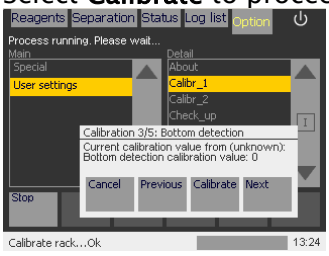


Figure 3.17 Select 'Calibrate' to continue with calibration. (Select 'Previous' or 'Next' to navigate through all five calibration steps).

2) Place a MACS Cooling Tube Rack onto the MACS MiniSampler. It is recommended to use a Chill 5 Tube Rack.

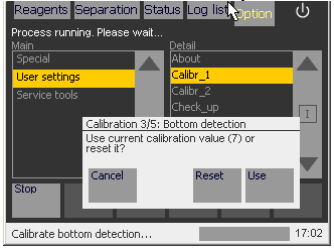
3) In the case of a Chill 5 Tube Rack, the needle will position itself a fraction of a millimeter over the surface of the Chill Rack at a point equidistant to the tube coordinates A1, A2, B1, B2. It should not make complete contact with the plastic surface.

Figure 3.18 Bottom detection calibration. In the case of a Chill 5 Tube Rack the needle will be positioned midpoint (equidistant) to tube coordinates A1, A2, B1, B2.

4) Select **Done**.

> **Note:** The uptake needle moves to the surface of the tube rack. The position of the needle tip is correct if a sheet of paper can be easily slid between the needle tip and the surface of the tube rack.

5) Select **Use** to start the calibration at current position (recommended). Select **Reset** to reset the position to factory settings.



6) To adjust the position, select **Change** or select **Done** if the current settings are correct.

7) If **Change** was selected (see above), use the **Up** and **Down** navigation buttons to adjust the position as required.

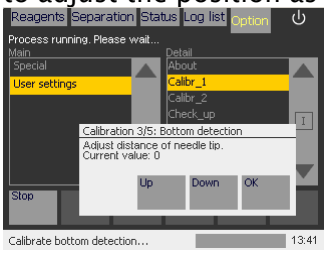


Figure 3.19 If necessary, adjust the z-axis of the needle tip using 'Up' and 'Down' navigation tools.

8) Select **OK** and **Done** to save the current configuration.

Calibration 1: Calibration of the x-axis – step 4/5

1) Select **Calibrate** to proceed with calibration.
2) Place a MACS Cooling Tube Rack and a MACS Reagent Rack 4 onto the MACS MiniSampler. It is recommended to use a Chill 5 Tube Rack.
3) Select **Done**.
4) Select **Use** to start the calibration at current position (recommended). Select **Reset** to use factory settings.

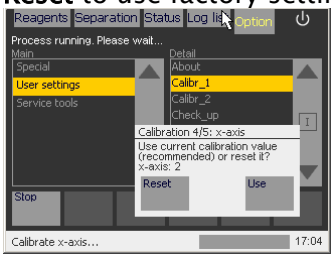


Figure 3.20 It is recommended to 'Use' the current calibration settings.

> **Note:** It is possible that the stored calibration value is zero!
> In this event, Figure 3.20 will not be displayed.

5) If necessary, adjust the central positioning of the uptake needle by using the needle navigation buttons (**Move left**, **Move right**, **Height**). The uptake needle must be positioned above the center of the tube in row A of the tube rack.

> **Note:** Due to mechanical limits of the MiniSampler the correction potential is limited. Exceeding these limits is reported by the message "standard range exceeded".
>
> Calibration outside the standard range is not recommended. The outer positions (reagent vial and sample row 6) will not be reached properly. Labeling in sample position 6 will be prohibited. In this event contact technical support (see section 12).

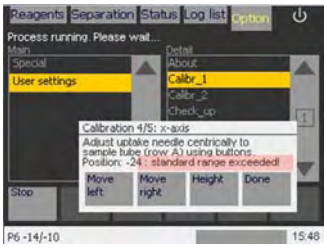


Figure 3.21 Adjust MiniSampler position

6) Select **Save** to store new settings.

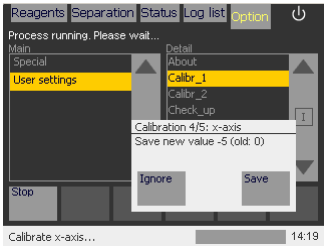


Figure 3.22 Select 'Save' to store new calibration settings.

Calibration 1: Test current calibration settings – step 5/5

1) Select **Test** to test new configurations. The autoMACS Pro Separator will perform a complete test of 'Calibration 1' settings.

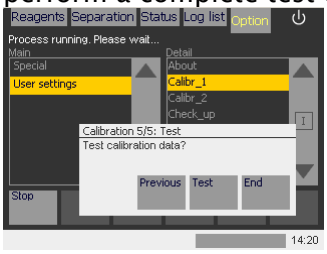


Figure 3.23 It is important to test the new calibration settings. To navigate back through the calibration steps, select 'Previous'.

2) If the test was successful select **End** to quit **Calibr_1**. If any errors or misalignments were noted, repeat the entire process.

Calibration 2

1) Select menu **Option**
2) Highlight **User settings** and select **Calibr_2**
3) Select **Run**.

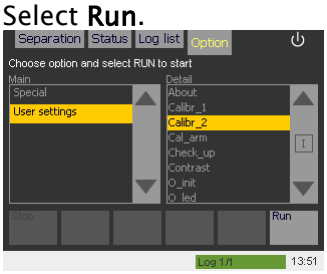


Figure 3.24 Performing 'Calibration 2' by selecting program 'Calibr_2'

4) Select **Calibrate**. The calibration is performed automatically.

5) Press **Save** to finish **Calibr_2**.

3.2.8 Installation of the autoMACS™ Columns

Note: When delivered, the autoMACS Pro fluidic system is filled with double-distilled water.

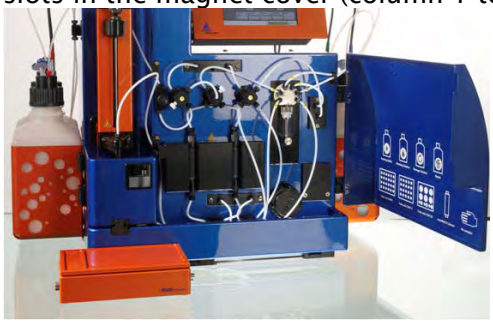
Note: The autoMACS Columns are reusable for 14 days or up to 100 separations, within 14 days whichever comes first.

Note: The instrument automatically records the date of the column exchange and can display the due date for the next column replacement if the program **Column exchange** (Col_ex) has been used for column installation. It is important to set the current time and date before exchanging the columns: select the **Option** menu from the upper navigation bar and **Set_time** from the **User settings** menu. Press **Run**. Enter the time in a 24-h-format using the numerical keypad. The date should be entered in the following format: dd-mm-yyyy. Press **OK**.

Note: Columns do not have a top or bottom and do not require special orientation in the column holder.

Remove the column substitutes and install the autoMACS Columns according to the following instructions:

1) Open the front door and note the position of the tubing and autoMACS Column slots in the magnet cover (column 1 to the left, column 2 to the right).



2) Ensure that the fluid containers are filled with solutions.

3) Using the touch screen, select **Option** and **Special** from the "Main" panel.

4) Select **Col_ex** (column exchange) from the "Detail" panel.

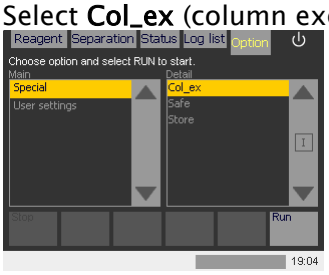


Figure 3.25 Selecting column exchange from the 'Option' tab.

5) Select **Run** to start program **Col_ex**. A popup dialog offers four options:
Remove to remove columns;
Install to remove and install new columns;
Sep count to inform the software how many separations have been performed;
Exit to cancel this process.

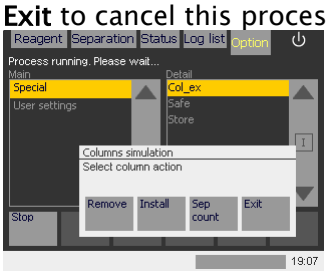


Figure 3.26 Select column action dialog box.

6) Select **Install** from the popup dialog box:

7) Wait until the instrument prompts you to exchange the autoMACS Columns before proceeding. Select **Done**.

> **Note:** Only install one column at a time.

8) Using both hands take the top and bottom of the column one substitute and pull gently but firmly to remove it from its slot.

9) Place a wide mouth container under the column substitute. Hold the column substitute in one hand and gently unscrew the upper column connector counter-clockwise. Tilt the column substitute to empty any fluid. Then, unscrew the bottom column connector. Store the column substitute in the autoMACS Pro Starting Kit box.

Figure 3.27 Removing column one for subsequent exchange.

10) Insert one end of a fresh column into the bottom column connector and gently screw in the column by turning it clockwise until you feel a resistance. Point the column towards the top of the device and screw in the top column connector.

11) Align the column with the top column connector sitting on the guiding of the magnet cover. Press the column into the slot until you feel the guides click. Verify that the column is placed in the center of the magnet cover.

12) Repeat steps 8 through 11 to install the second column.

13) Ensure that the tubes are securely fastened to the columns and that the tubing is not pinched or obstructed.

14) Close the front door. Press **Done**. The unit is now ready to perform cell separations.

> **Note:** The program will then proceed to wash the columns with autoMACS Running Buffer. Check that the column is securely fastened to the column connectors and that no buffer is leaking.

3.2.9 Priming the autoMACS™ Pro Separator

Priming implicates the initial cleaning and filling of the autoMACS Pro Separator tubing system before cell separations are performed. The autoMACS Pro Separator must be primed each time the instrument is switched on.

> **Note:** Read the warnings and precautions section before priming the autoMACS Pro Separator for the first time.

1) Fill all bottles with the appropriate solutions and empty the waste bottle.

> **Note:** When working with biohazardous samples, it is recommended to fill the waste container with 100 mL of disinfectant before use (e.g. MACS Bleach solution). For proper disposal, please follow local regulations and carefully read the chapter Important information.

2) Switch on the instrument. After initialization is completed, the **Status** menu will be displayed. Verify that the touchscreen symbols for all fluid containers are green. If this is not the case, check if the fluid sensor cables are connected to the correct fluid containers. The symbol for the ethanol container remains gray (fluid level not checked by system). At this point, the bottle illumination is yellow.

Figure 3.28 The status menu. The fluid level status and column are green for 'go'. A wash or instrument prime has not yet been performed.

3) Select menu **Separation** and **Wash Now** from the lower menu. You now have the option to perform a quick rinse (**Qrinse**) or full rinse (**Rinse**).

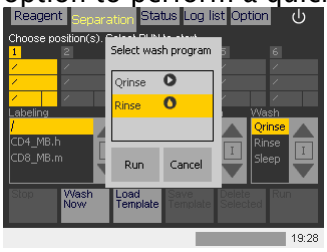


Figure 3.29 Select 'Rinse' to prime the autoMACS Pro Separator.

4) Select **Rinse** and **Run** to start the priming process. The progress will be displayed at the bottom of the touchscreen menu.

5) When priming is finished, the instrument will display **Ready for separation** in the **Status** menu. The bottles are illuminated green.

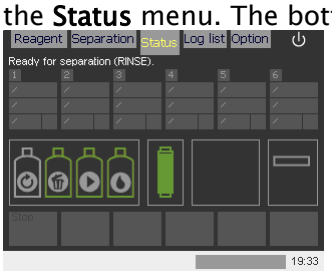


Figure 3.30 The instrument has been primed (rinsed).

> **Note:** A cell separation template can already be programmed or reloaded during the priming procedure. By selecting **Run**, the cell separation will be started immediately after priming is finished.
>
> **Note:** The autoMACS Pro Separator will display a warning screen if the buffer supply is low or if the waste bottle is full. If no wash program has been performed before the first separation, a warning screen will ask to rinse the system.
>
> **Note:** You may interrupt any program by selecting **Stop**. The **Stop** button is located at the bottom left hand corner in all menus.
>
> **Note:** When priming the instrument for the first time or when the instrument was not serviced for a long period of time, it is recommended to visually inspect the fluidic system for potential leaks. Open the front door after priming the instrument. If there is any sign of leakage, tighten the respective tubing connection. Close the front door and proceed with the separation.

3.2.10 Checking the fluid levels

The autoMACS Pro Separator automatically ensures that the uptake port is filled with buffer, that fluid containers carry enough fluid for one separation, and that the waste container can collect fluid from at least one separation. If more than one separation is performed, ensure that the containers contain sufficient fluid for all the separations and that the waste container is empty.

To verify the status of the bottles, select the menu **Status** from the upper navigation bar. On the left hand side of the menu, four symbols display the solution containers and their filling status. If the fluid containers are full and the waste container is empty, the symbols are green. If the solution containers are empty or the waste container is full, the respective symbols are red. The symbol for the ethanol container remains gray. It is recommended to select the menu **Status** when the autoMACS Pro Separator is in operation.

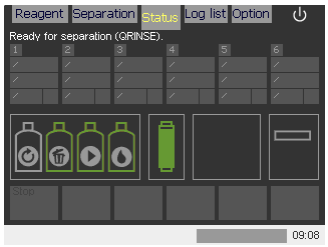


Figure 3.31 Status of the fluid containers is green for ready.

3.2.11 Installation of the 2D code reader (barcode reader)

Note: Carefully read the chapter important information before installing the 2D code reader.

Note: If the autoMACS Pro Separator is not provided with the optional 2D code reader, an autolabeling package is required for installation. Please contact Miltenyi Biotec or your local distributor for further information. The autolabeling package contains:
1 x 2D code reader
1 x MACS Reagent Rack 4
1 x Autolabeling Software
Tools for installation (a 2 mm allen key, a headless screw, an optical frame for the 2D code reader window in the Washing Station cover and a screw driver for connecting the reader to the outlet at the rear of the instrument).

1) Switch off and unplug instrument.

2) Open and remove the Washing Station Cover. Remove the peristaltic pump tube at the right hand side of the washing station.

Figure 3.32 Remove the peristaltic pump tube

3) Unscrew the thumb screw. Pull to remove washing station from device. Take care to clean spilled fluids with ethanol or disinfectant. Remove the washing station.

Figure 3.33 Unscrew the thumb screw

4) Note the position of the 2D code reader Port.

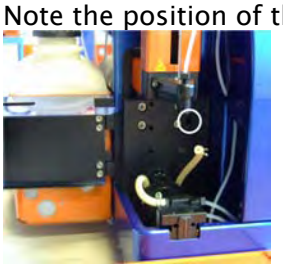


Figure 3.34 the position of the 2D code reader port

5) Guide the cable of the 2D code reader underneath the lower front access cover at the left-hand side. Make sure that 2D code reader cable is located in-between the peristaltic pump tubes.

Figure 3.35 Make sure that 2D code reader cable is located in-between the peristaltic pump tubes

6) Insert 2D code reader pin into corresponding opening.

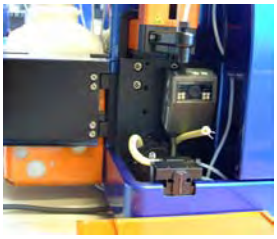


Figure 3.36 Inserted 2D code reader

7) Fix the connection by fastening the headless screw in the tapped bore hand-tight with help of a 2 mm allen key.

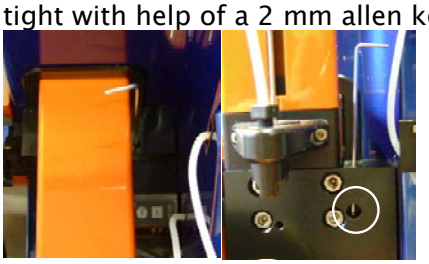


Figure 3.37 Left: Fastening with allen key. Right: Screw not present

> **Note:** Please check if headless screw is already present in the tapped bore. Otherwise use headless screw provided with autolabeling package.

8) Re-install the Washing Station. Make sure that the cable is installed straight and is guided between the peristaltic pump tubing.

Figure 3.38 Re-install Washing Station

9) Remove the black plastic square from the 2D code reader window of the Washing Station Cover (not shown). Snap in black optical frame. Re-install and close the Washing Station Cover.

10) Guide the 2D code reader cable underneath the instrument. Connect the 2D code reader plug with the corresponding outlet at the back of the device labeled “RS232/BCR”. Use the screw driver to fasten the screws of the connecter.

11) Plug in and switch on instrument.

12) Select menu **Option**. Highlight **User Settings** and **O_bcr**. Press **Run**.

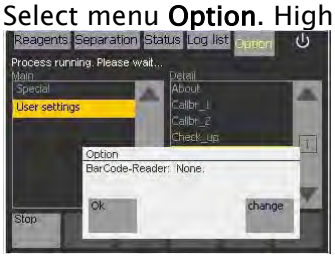


Figure 3.39 Setting up the software to recognize the 2D code reader.

13) Select **Change**.

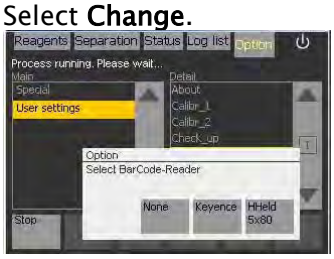


Figure 3.40 Select ‘Change’ to recognize the recently installed 2D code reader.

14) Select **Keyence**.

15) Switch off instrument. Wait 3 seconds and switch on instrument again.

4 An overview of the autoMACS™ Pro touchscreen user interface

The autoMACS Pro Separator is operated through a TFT color touchscreen. After switching on the instrument, the start screen will appear.

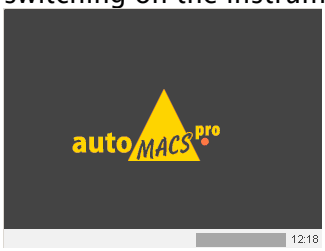


Figure 4.1 The autoMACS Pro Separator startup screen

4.1 The main menu screen

Five main menus allow easy interaction with the instrument. They are accessed through the upper tabbed menu:

Tab	Function
Reagent:	To define the position of MACS Reagents on the MACS Reagent Rack 4.
Separation:	To define the autolabeling and cell separation strategy for up to six samples. In addition, cell processing procedures can be saved as templates for regular use.
Status:	The instrument status is displayed at a glance.
Log list:	The log list details completed actions and errors.
Option:	Users can perform special procedures such as exchange of MACS Columns, instrument calibration and service steps.

Table 4.1 Feature overview of the upper tabbed menu.

After instrument initialization the screen automatically displays the **Status** menu, which helps to monitor the instrument during installation and operation. Color-coded symbols indicate the status of the hardware components. Further information on a particular component can be obtained by touching the symbol.

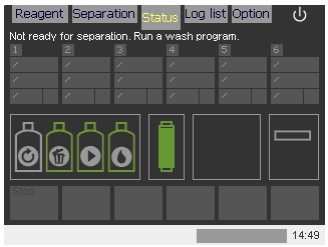


Figure 4.2 The main 'root' menu screen displays instrument 'Status' at startup.

4.1.1 Working with the lower menu bar

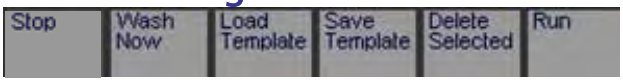


Figure 4.3 The lower menu bar of the 'Separation' menu.

A lower menu bar is accessible from all **Menu** screens. Depending on the status of the instrument and the selected upper tabbed menu option, the lower menu buttons switches between an inactive (grey background) and active state (white background) state. For example, **Stop** can only be selected when the instrument is actively performing a process such as cell separation.

Figure 4.4 Left: Stop button is active and can be selected. Right: Stop button is inactive and cannot be selected.

4.1.2 Reagent menu

The reagent menu is used to program any reagent vials that are required for automated magnetic labeling and subsequent cell sorting. Reagents can be entered using the 2D code reader or manually using the **Enter Reagent** input panel.

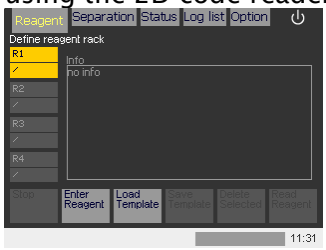


Figure 4.5 The reagent menu

Entering reagent information for autolabeling

Reagents vials can be directly scanned and recognized by the autoMACS Pro Separator 2D code reader.

Scanning reagents with the 2D code reader

1) Select **Reagent** tab and highlight the position where the vial will be placed on the reagent rack. Four positions are available: **R1**, **R2**, **R3** and **R4**.

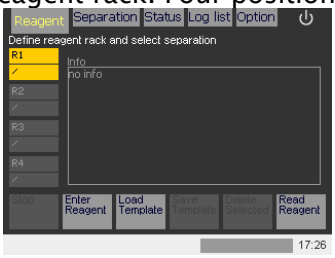


Figure 4.6 Position R1' was selected for the reagent vial.

2) Activate the reader by selecting **Read Reagent**, 

and present a reagent vial in front of the 2D code reader. Ensure the 2D code is facing the blinking code-reader light. The optimal reading distance is 0.5–2.5 cm from the code reader cover, tilt the vial as depicted in Figure 4.7.

Figure 4.7 Scanning a reagent vial using the 2D code reader. In this example the vial "CD4 Microbeads, human" was scanned.

3) The vial is automatically recognized by the software. The next reagent rack position will be automatically highlighted (**R2**).

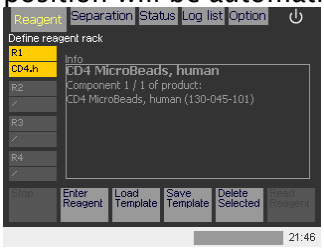


Figure 4.8 CD4 MicroBeads, human (product number 130-045-101) was identified by the 2D code reader software and assigned to the reagent rack position 'R1'.

> **Note:** If the reagent vial cannot be identified by the 2D code reader please enter the reagent information manually; see "Entering reagents manually" below.

4) Using the same procedure another reagent vial can be scanned using the 2D code reader. The reagent rack position R3 is automatically assigned. Having entered the desired reagent(s), click the **Separation** tab to proceed with programming a cell separation.

Entering reagents manually

1) Select **Reagent** tab and highlight the position where the vial will be placed on the reagent rack. Four positions are available: **R1**, **R2**, **R3** and **R4**.

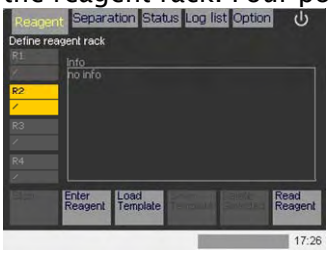


Figure 4.9 Position 'R2' was selected for the reagent vial.

2) Select **Enter reagent** from the lower navigation bar. Enter the reagent-specific product order number. The order number is located on the product data sheet. In the event that the data sheet is misplaced, visit www.miltenyibiotec.com to download a printable PDF of the document.

Figure 4.10 Reagent information is manually entered using the reagent order number.

3) Select **OK**.

4) If a correct number is inserted the software will immediately recognize the reagent or kit. To confirm, select the reagent from the list by using the touch screen.

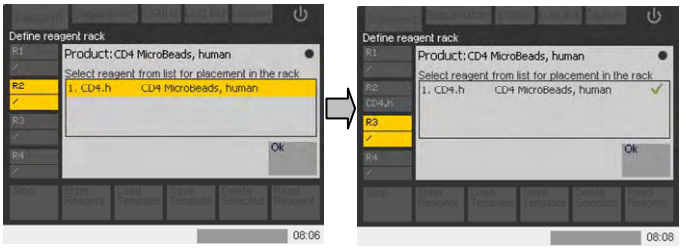


Figure 4.11 CD4 MicroBeads, human (product number 130-045-101) was manually entered.

5) Select **OK** to confirm the identified reagent and reagent vial position **'R2'**.

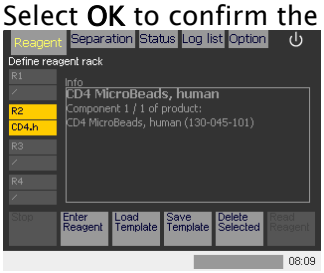


Figure 4.12 Reagent vial "CD4 MicroBeads, human" was assigned to the reagent rack position 'R2'.

Deleting reagents or reagent lists

In this example the following reagents were scanned into the following reagent rack positions:

- R1: FcR Blocking Reagent, human (component 1/3 of product Monocyte Isolation Kit II, human; product number: 130-091-153)
- R2: Monocyte Antibody Cocktail , human (component 2/3 of product Monocyte Isolation Kit II, human; product number: 130-091-153)
- R3: Anti-Biotin MicroBeads (CX) (component 3/3 of product Monocyte Isolation Kit II, human; product number: 130-091-153)
- R4: CD4 MicroBeads, human; product number: 130-045-101

To delete an individual reagent from the reagent rack list:

1) Select the reagent name that must be deleted. In this example **CD4 MicroBeads, human** on position **R4** must be removed.
2) Select **Delete Selected** to remove the highlighted reagent.

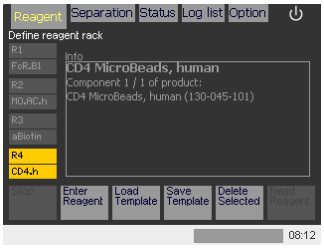


Figure 4.13 CD4 MicroBeads, human was assigned to reagent rack position R4, which was subsequently selected for deletion.

To delete the entire reagent list:

1) Select an unassigned position on the reagent rack. In this example position R4 was selected. (If there are no unassigned positions, delete an individual reagent as described above).

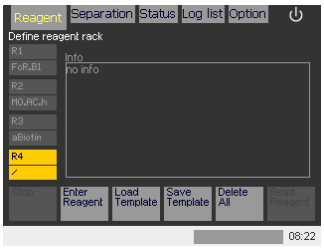


Figure 4.14 To delete all reagents from the reagent rack, an unassigned position on the reagent rack must be highlighted. In this example the only unassigned position 'R4' was selected.

2) Select **Delete All**.

Working with reagent templates

For convenience it is possible to load and save reagent templates. In this example the following reagents were scanned into the following reagent rack positions:
R1: CD4 MicroBeads, human; product number: 130-045-101
R2: CD8a (Ly-2) MicroBeads, mouse; product number: 130-049-401
R3: No reagent
R4: No reagent

To save a reagent template:

1) Assign reagent vials to reagent rack positions as outlined above (4.1.2).

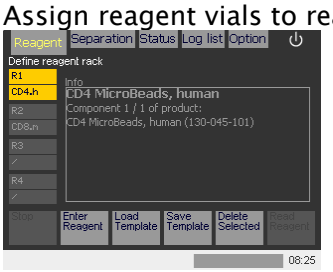


Figure 4.15 Using the 2D code reader, two reagent vials were assigned to the reagent rack positions 'R1' and 'R2'.

2) Select **Save Template**.

3) Allocate a name to the template. In this example the template was saved as "EXPT_2" (experiment 2).

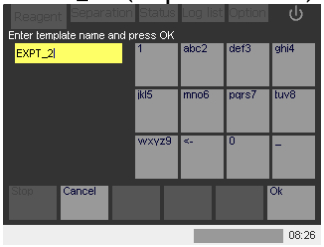


Figure 4.16 Using the alphanumeric keypad assign a name to the template.

4) Select **Ok**.

To load a reagent template:

1) Select **Load Template** from the lower navigation bar. To scroll through the list of saved templates use the navigation arrows,  . The corresponding template is displayed in the adjacent panel.

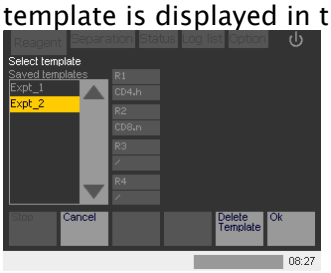


Figure 4.17 Selecting a reagent template. Expt_2 was selected which comprises of CD4.h and CD8.m on positions R1 and R2, respectively.

2) Select and highlight the desired template; in this case “Expt_2”

3) Select **Ok**.

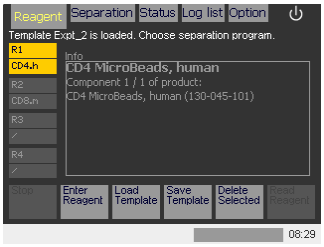


Figure 4.18 The template Expt_2 was successfully loaded.

To delete a reagent template:

1) Select **Load Template** from the lower navigation bar.

2) Scroll through the list of saved templates using the navigation arrows,  .

3) Select the template for deletion.

4) Select **Delete Template**.

4.1.3 Separation menu

The **Separation** menu schematically represents a sample rack and allows definition of sample processing strategies for each sample rack position. For each sample rack position it is possible to define cell labeling, cell separation and washing programs. A single wash program can be performed by selecting **Wash Now** from the lower navigation bar.

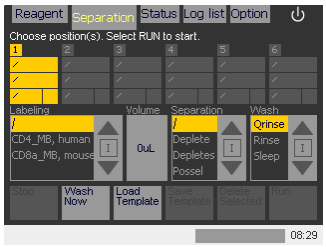


Figure 4.19 The separation menu.

An overview of the separation menu follows:

1 Sample rack template
2 Sample labeling options
3 Sample processing volume
4 Separation program
5 Wash procedure

Sample rack template

The positions in the programming field (1) correspond to the sample positions in the tube rack. Positions 1–6 are used in combination with tube rack Chill 5, positions 1–5 with rack Chill 15, and positions 1–3 with rack Chill 50.

To select/deselect a sample position touch to highlight the desired position 1–6.

1) Select/deselect a sample position by touching the display.

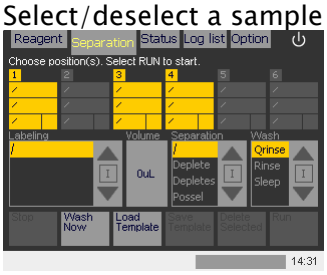


Figure 4.20 Sample positions 1, 3 and 4 were selected.

Sample labeling options

The **Labeling** submenu (2) is used to instruct the instrument:

- If autolabeling is to be performed on a sample.
  The default setting is **'/',** which indicates that NO autolabeling will be performed.
- The type of autolabeling that will be performed.
  A list of product options is only visible if reagents have already been assigned to positions on the reagent rack using the **Reagent** menu.

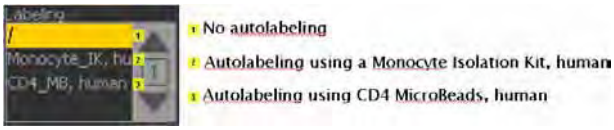


Figure 4.21 'Labeling' submenu. Using the MACS Reagent Rack 4, a total of four reagents may be used at any given time.

Assigning sample volumes

The **Volume** submenu (3) is used to inform the instrument about the available sample volume. To enter or modify a volume:

1) Ensure that the appropriate sample template position is highlighted.
2) Select the **Volume** submenu.
3) Using the numeric keypad enter the sample volume. For autolabeling enter the volume for the first labeling step as outlined in the corresponding data sheet (e.g. 160 µl / $2x10^7$ cells for labeling with $CD4^+$ MicroBeads, human or 120 µl / $4x10^7$ cells for labeling with Monocyte Isolation Kit II, human).

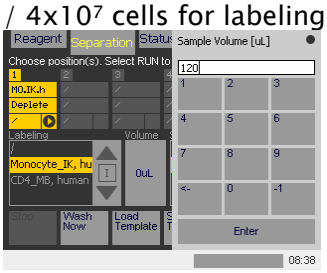


Figure 4.22 Entering the sample volume in microliters for Monocyte Isolation Kit II, human.

4) Select **Enter**.

Assigning a cell separation program

The **Separation** submenu (4) is used to instruct the autoMACS Pro Separator which cell separation program should be applied to each sample. Several sample positions can be highlighted to assign a separation condition for multiple samples. Refer to section 6.2.1 for a detailed explanation of the various cell separation strategies. To assign or modify a cell separation program:

1) Highlight the appropriate sample(s) using the sample template.
2) Scroll through the **Separation** submenu using the arrows  .
3) Select and highlight a **Separation** program .

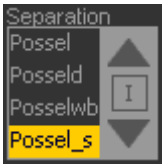


Figure 4.23 Positive cell separation-sensitive mode was selected.

Assigning a wash program

The **Wash** submenu (5) is used to instruct the autoMACS Pro Separator which wash program should be applied to each sample. Three wash programs are available to choose from:

- Qrinse (quick rinse) –  : Recommended to save time between sample separation steps. The tubing and column receive a quick rinse.
- Rinse –  : The system is 'primed', receiving an extensive wash. **Rinse** is recommended between rare cell and whole blood separations.
- Sleep –  : A rinse is performed before the system is shutdown.

To assign or modify a wash program:

1) Highlight the appropriate sample(s) using the sample template.
2) Scroll through the **Wash** submenu using the arrows  .
3) Select (highlight) a **Wash** program.

Figure 4.24 A rinse program was selected.

Working with Separation templates

For convenience it is possible to load and save separation templates. **Separation** templates can be used in combination with **Reagent** templates (see above).

To save a separation template:

1) Configure the sample template, an example follows.

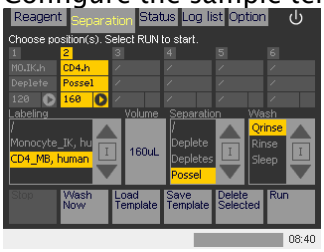


Figure 4.25 A sample template was setup to process 2 samples.
Sample 1: Isolation of untouched human monocytes using the Monocyte Isolation Kit II (130-091-153); autolabeling program MO.IK.h; separation program 'Deplete'.
Sample 2: Direct labeling and positive cell enrichment of CD4+ cells using CD4 MicroBeads, human (130-045-101); autolabeling program CD4.h; separation program 'Possel'.

2) Select **Save Template**.

3) Allocate a name to the template. In this example the template was saved as "EXPT_2A" (experiment 2a).

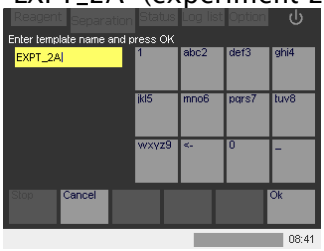


Figure 4.26 Using the alphanumeric keypad assign a name to the template.

4) Select **Ok**.

To load a reagent template:

1) Select **Load Template** from the lower navigation bar. To scroll through the list of saved templates use the navigation arrows,  /  . The corresponding template is displayed on the sample template panel.

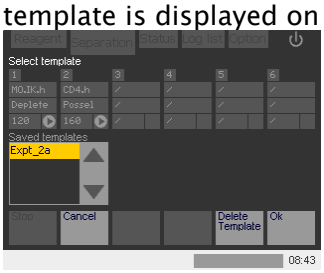


Figure 4.27 Selecting a separation template. Expt_2a was selected.

2) Select and highlight the desired template; in this case "Expt_2a"

3) Select **Ok**.

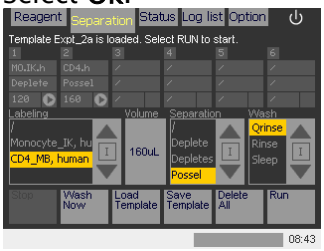
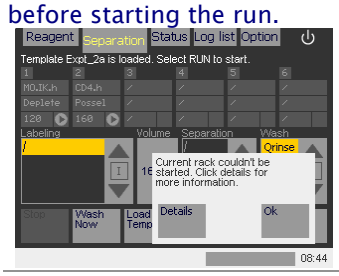


Figure 4.28 The template Expt_2a was successfully loaded.

Note: The sample separation template was loaded; however, the reagent rack has not yet been configured. It is necessary to configure the reagent rack before starting the run.



To delete a Separation template:

1) Select **Load Template** from the lower navigation bar.
2) Scroll through the list of saved templates use the navigation arrows,  .
3) Select the template for deletion.

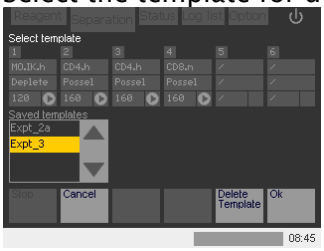


Figure 4.29 Expt_3 template was selected for deletion.

4) Select **Delete Template**. Select **Delete Template**.

4.1.4 Status menu

The autoMACS Pro Separator is a sensor-controlled device that allows easy monitoring during operation. At a glance the instrument status can be determined by viewing the **Status** menu.

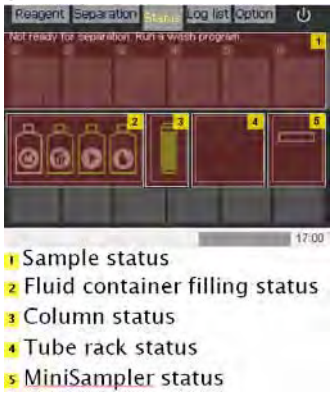


Figure 4.30 Overview of the instrument status panels.

A description of how to monitor the instrument's status using the **Status** menu follows.

Status of fluid containers

Figure 4.31 Fluid container status symbols. Left: Fluid containers are ready. Right: Fluid containers need replaced.

The status of fluid containers is indicated by color-coded graphic symbols and by a text table.

Container	Symbol	Symbol color and user action
Running Buffer		Green: No action required Red: Refill container Gray: Connect bottle sensor
Washing Solution		
Storage Solution		Gray: No liquid detection; visually check volume
Waste		Green: No action required Red: Empty waste Gray: Connect bottle sensor

Table 4.2 Status of fluid containers displayed in the 'Status' menu

In addition to color-coded graphic symbols of the fluid containers, a popup text table also reports fluid container status. Touch any fluid bottle symbol to active the popup textbox. To close the popup table, select  . Moreover, the symbols are also red, when the sensor cables are connected to a wrong bottle closure

Figure 4.32 Fluid container status. By touching any fluid container symbol a popup box displays a text report of the fluid container status.

Column status

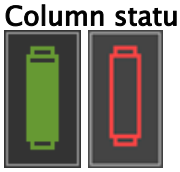


Figure 4.33 The column status graphic. Left: The column is ready. Right: The column must be changed.

If the column symbol is green, no action is required. If the symbol is red the columns must be exchanged. The level of the green fill on the column symbol indicates the remaining service-life of the autoMACS Columns. Touch the column symbol to activate the column status popup textbox.

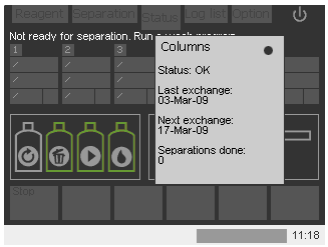


Figure 4.34 Column status. By touching the column symbol a pop-up box displays a text report of the column status.

Tube rack status

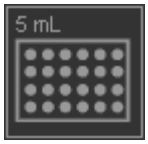


Figure 4.35 Tube rack status graphic: a 5 mL tube rack was detected. If no tube rack is detected no graphic is displayed.

Rack detection occurs prior to starting the separation process. Before cell labeling and/or cell separation is performed the instrument will not attempt to detect the rack. Touch the position of the rack status graphic in order to view the tube rack popup text table.

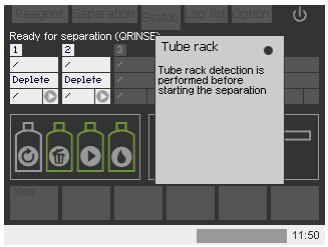


Figure 4.36 Tube rack status text box: No rack is detected until cell separation is performed.

MACS MiniSampler status

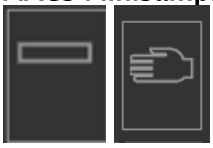


Figure 4.37 MACS MiniSampler status graphic. Left: The MiniSampler was successfully installed. Right: No MiniSampler was detected.

If the MACS MiniSampler has been detected correctly a rectangular symbol is displayed. If it has not been detected a hand symbol will be displayed in the same field.

Sample status

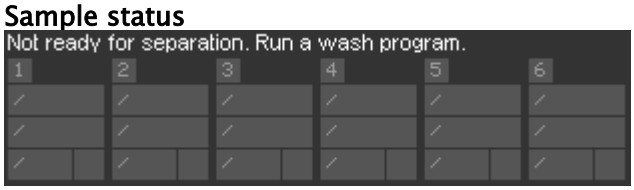


Figure 4.38 The sample status panel before performing cell separation.

It is recommended to monitor the instrument's status during cell labeling and/or separation using the **Status** menu. Program statuses are displayed using color-coded graphics. An overview of the colored symbols is tabulated below.

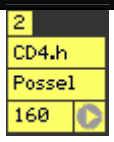
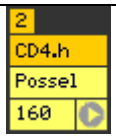
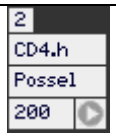
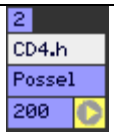
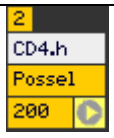
	Graphic	Definition		Graphic	Definition
1		Status: Waiting. Sample processing has not yet started.	4		Rinsing.
2		Sample autolabeling is underway.	5		Sample processing is completed.
3		Incubation of cells with labeling reagents.	*		Progress has been stopped or cancelled.
4		Sample is being processed, e.g., sample uptake.			

Table 4.3 Color-coded sample status graphics. CD4 MicroBeads autolabeling and positive cell enrichment was performed to demonstrate the sample status graphics.

> **Note:** The status bar, located below the lower navigation bar, always reports the exact status of the instrument. For example, during autolabeling the lower status bar reports: "Autolabeling…"

Figure 4.39 The lower status bar reports the current instrument action – this is reported by text and a progress bar graphic. Left: Autolabeling is being performed; the progress bar indicates a 2 minute process. Right: Sample Uptake is being performed; the progress bar indicates a 1 minute process.

Moreover, by selecting a sample position using the touchscreen, further details about the exact process is reported by a popup textbox.

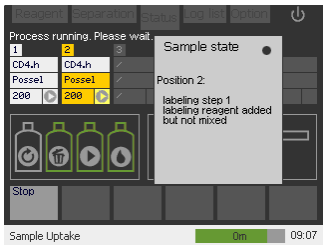


Figure 4.40 To find out more details about the sample state, touch the sample status panel to activate the 'Sample state' popup textbox. In this example sample position 1 has been completed; an aliquot of labeling reagent has been added to the sample at position 2, however the sample has not yet been mixed.

To summarize, a program currently in progress is depicted in orange whereas completed programs are depicted in white. Programs depicted in violet indicate sample incubation with labeling reagent.

4.1.5 Log list menu

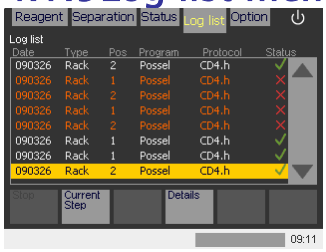


Figure 4.41 The 'Log list' display.

The **Log list** records a complete log of actions performed by the autoMACS Pro Separator. An overview of the log list table follows:

Name	Definition	Name	Definition
Date	Indicates the date the action was performed.	Program	Name of program, for example: **Deplete**: Cell depletion. **Possel**: Positive cell selection. **Qrinse**: Quick rinse performed.
Type	Description of the action, for example: **Rack**: Action involving rack **Init**: Initiation of instrument. **Wash only**: Wash only performed. **Service**: Service step performed. **Special wash**: Special wash performed.	Protocol	If a protocol is associated with the log, its name will be listed under this heading, for example: **CD4.h**: CD4 MicroBeads, human were used for autolabeling and cell separation.
Pos	Corresponding position on sample tube rack.	Status	The status of the log is depicted as follows: ✅ = successfully completed ❌ = action failed

To display further details about an individual log:

1) Select **Log list** tab and highlight a log from the log list.

2) Select **Details**. A detailed view of the program status is shown.

3) Select **Ok** to return to the **Log list** screen or select **Log Details** to view a detailed log of performed actions.

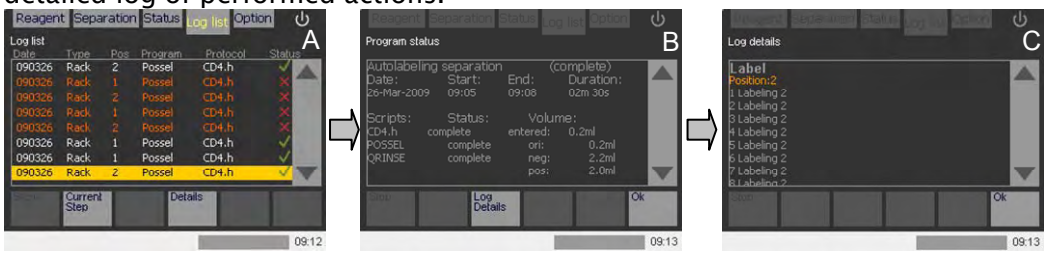


Figure 4.42 Viewing log details.
A: A CD4 MicroBead positive cell selection was performed on March, 26th 2009.
B: An overview of program actions and corresponding times are displayed.
C: Details of each instrument action are listed.

4.1.6 Option menu

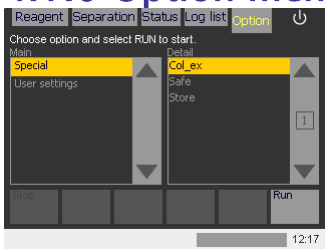


Figure 4.43 The 'Option' display.

The **Option** menu allows maintenance procedures, such as exchange of autoMACS Columns or decontamination of the system. The menu is divided into two main categories, **Special** and **User settings**. An overview of the functions available under each category are given below.

Special

Special options comprise three 'special' programs for column exchange (**Col_ex**), instrument decontamination (**Safe**) and cleaning of the device for long-term storage (**Store**).

Figure 4.44 Available 'Special' options.

Each program is briefly discussed below.

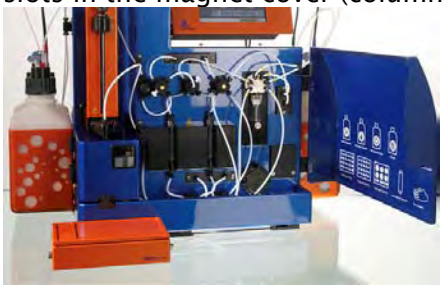
Column exchange: Col_ex

This program must be used to perform a column exchange.

Note: By touching the column status symbol, the date of the last column exchange, the due date for the next column exchange, and the number of separations performed with the currently installed columns are displayed in a pop-up screen (Column status). If the symbol for the columns is red, the columns must be exchanged (Figure 4.33).

Exchange the autoMACS Columns according to the following instructions:

1) Open the front door and note the position of the tubing and autoMACS Column slots in the magnet cover (column 1 to the left, column 2 to the right).



2) Ensure that the fluid containers are filled with solutions.

3) Using the touch screen, select **Option** and **Special** from the **Main** panel.

4) Select **Col_ex** (column exchange) from the **Detail** panel.

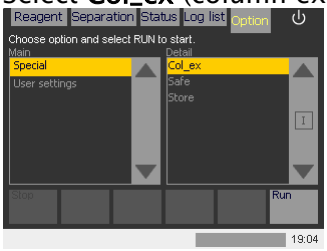


Figure 4.45 Selecting column exchange from the 'Option' tab.

5) Select **Run** to start program **Col_ex**.

6) Wait until the instrument prompts you to exchange the autoMACS Columns before proceeding. Select:
Remove to remove columns;
Install to remove and/or install columns;
Sep count to inform the software how many separations have been performed;
Exit to cancel this process.

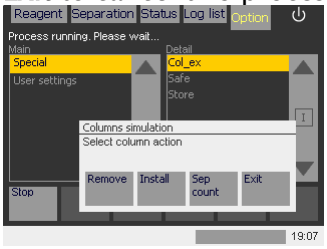


Figure 4.46 Column exchange; select 'Install' to remove and/or install new columns.

7) Select **Install**.

Note: Only install one column at a time.

8) Using both hands take the top and bottom of column one substitute and pull gently but firmly to remove it from its slot.

9) Place a wide mouth container under the column substitute. Hold the column substitute in one hand and gently unscrew the upper column connector counter-clockwise. Tilt the column substitute to empty any fluid. Then, unscrew the bottom column connector. Store the column substitute in the autoMACS Pro Starting Kit box.

Figure 4.47 Removing column one for subsequent exchange.

10) Insert one end of a fresh column into the bottom column connector and gently screw in the column by turning it clockwise until you feel a resistance. Point the column towards the top of the device and screw in the top column connector.

11) Align the column with the top column connector sitting on the guiding of the magnet cover. Press the column into the slot until you feel the guides click. Verify that the column is placed in the center of the magnet cover.

12) Repeat steps 8 through 11 to install the second column.

13) Ensure that the tubes are securely fastened to the columns and that the tubing is not pinched or obstructed.

14) Close the front door. Press **Done**.

Safe

This is a disinfectant procedure which uses MACS Bleach for cleaning and decontamination of the autoMACS Pro Separator. Depending on the level of use and general instrument maintenance, it is recommended to decontaminate the fluidic system every 3 to 6 months using the **Safe** program.

Perform instrument decontamination according to the following instructions:

1) Select menu **Option**, **Special** and the program **Safe**.

2) Press **Run**. Follow the screen prompts.

Figure 4.48 'Safe' program is underway.

3) Disconnect the tubings from all buffer bottles. Select **OK.**

4) Place the end of each tubings into a canister of MACS Bleach solution.

5) The decontamination procedure is performed automatically. Upon completion of the process, replace fluid containers and reconnect all tubings.

6) Select **OK**. The system is rinsed automatically.

Store

The program **Store** should be applied to prepare the instrument for long-term storage. Upon completion of the **Store** program, the fluidic system contains 70% ethanol.

To store the autoMACS Pro Separator for a period longer than two weeks, the tubing system should be cleaned and the columns should be replaced with column substitutes. The **Store** program automatically performs the cleaning procedure and prompts the user to install column substitutes.

1) Select the **Option**, **Special** and **Store**. Select **Run**. The system will be rinsed automatically.

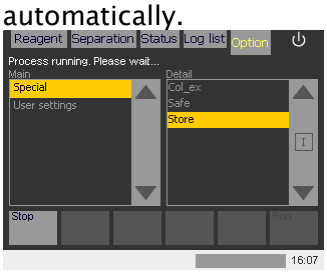


Figure 4.49 'Store' program is underway.

2) Install the column substitutes as described by the section 3.2.8.

3) Select **Done**.

4) Switch off the autoMACS Pro Separator using the main power switch.

User settings

The **User settings** are for maintenance and setup of the autoMACS Pro Separator. Each program is briefly discussed below.

About

About informs the user about the software version, serial number of the instrument and other hardware information.

1) Select the **Option** menu tab, **User settings** and **About**.

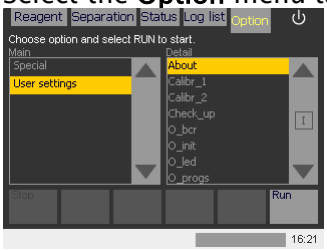


Figure 4.50 Viewing 'About' information

2) Select **Run**.

3) After viewing the device information, select **OK** to return to the previous menu,

Calibr_1: Performing "calibration 1" of the needle arm positioning

This program is used for the calibration of the needle arm to the washing station and tube racks. See section 3.2.7 for more information.

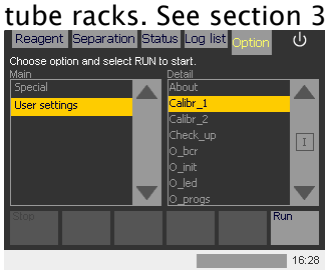


Figure 4.51 Performing calibration 1.

Calibr_2: Performing "calibration 2" of the device tubing

This program is used for the calibration of the device tubing. See section 3.2.7 for more information.

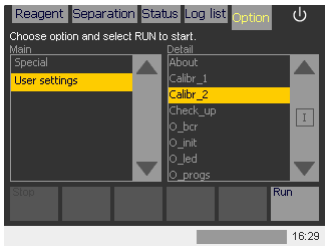


Figure 4.52 Performing calibration 2.

Check_up: Performing a system check-up

The **Check_up** program allows the user to perform a system check-up. It is recommended to use the program if hardware errors occur. The program starts after highlighting **check_up** and pressing **Run**.

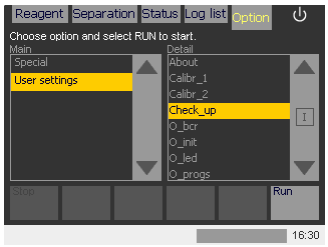


Figure 4.53 The check_up program

O_bcr: 2D code reader setup, configuration and initialization

The O_bcr program allows the user to setup, configure and initialize a recently installed autoMACS Pro Separator 2D code reader.

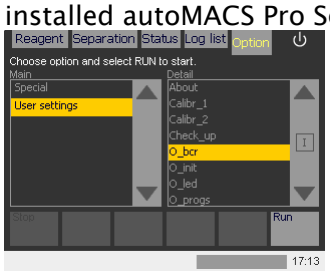


Figure 4.54 O_bcr program for initialization and setup of an installed 2D code reader.

> **Note:** This program should only be used in combination with the autoMACS Pro Upgrade Kit.

O_init: Optional priming of the instrument at startup

By default, the autoMACS Pro Separator does not perform a wash program after initialization. The option **O_init** allows the user to add an initial **Rinse** program that will be performed automatically after each initialization to prime the instrument.

1) Select **Option**, **User settings** and **O_init**. Press **Run**.
2) Follow the prompt on the screen to enable or disable the initial wash.

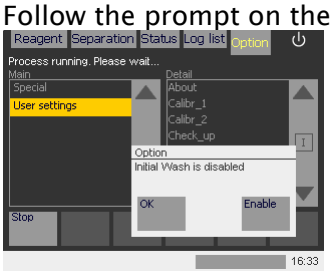


Figure 4.55 Initial Wash program is used to activate or deactivate an automatic instrument prime on instrument startup.

> **Note:** The initial **Rinse** program may interfere with the rescue procedure (described in section 7.16) that can be performed after disruption of the power supply during cell separation. The program **Rinse** utilizes Washing Solution containing a detergent which would have deleterious effects on the cells during the rescue procedure. To avoid this, follow the instructions described in section 7.16.

O_led: Activating/deactivating bottle illumination

The autoMACS Pro Separator has a bottle illumination designed to facilitate monitoring the instrument's status. The bottle illumination can be switched ON or OFF.

1) Select **Option**, **User settings** and **O_led**.
2) Select **Run**.
3) Follow the screen prompts to **Enable** or **Disable** bottle illumination.

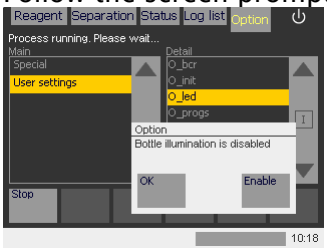


Figure 4.56 Activating or deactivating bottle illumination.

O_progs: Enabling/disabling special separation protocols

The **O_progs** are used to enable or disable special separation programs. To enable or disable these protocols, perform the following steps:

1) Select **Option**, **User settings** and **O_progs**.
2) Select **Run**. A dialog box will report the current status.

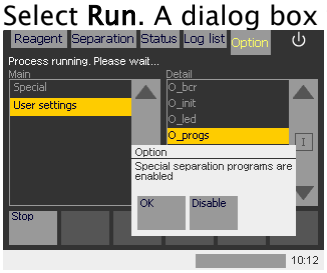


Figure 4.57 O_progs: Enabling or disabling special separation protocols. Select 'Disable' to disable special separation programs.

3) Select **OK** if the current reported status should not be changed. Alternatively, select **Disable** (or **Enable**) to change the status.

Sepcount: Displaying the number of performed column separations

The **Sepcount** or "Sep. counter" program is used to display the number of column separations that have been on the autoMACS Pro Separator. To view this statistic, perform the follows steps:

1) Select **Option**, **User settings** and **Sepcount**.

2) Select **Run**. A dialog box will report the number of separations.

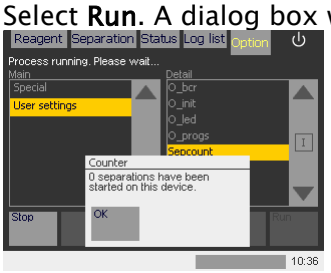


Figure 4.58 Sepcount: viewing the number of performed separations. In this example, no separations have been performed using this device.

3) Select **OK** to return to the **Option** menu.

Set_time: Setting the time and date

To set the time and date perform the following steps:

1) Select **Option**, **User settings** and **Set_time**. Select **Run**.

2) Highlight either the **time** or **date** fields by touching the display.

3) Use the keypad to change the date or time accordingly.

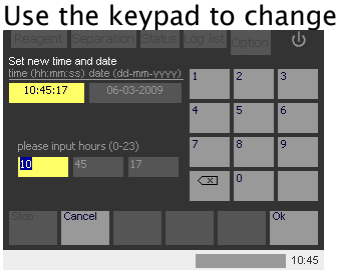


Figure 4.59 Select the desired field and use the numeric keypad to modify the date and/or time.

4) Select **OK** to return to the **Option** menu. The procedure can be cancelled at anytime by selecting **Cancel**.

Valve_ex: Exchanging the instrument values

This program is used for valve exchange. Use of this program turns the valve to the 'exchange' position for removal. Valves may require periodical exchange. See section 7.5 for more details.

To set values to the exchange position:

1) Select **Option**, **User settings** and **Valve_ex**. Select **Run**.

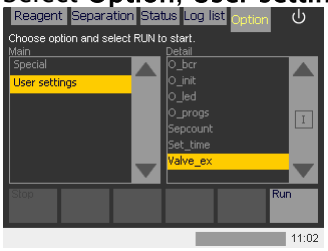


Figure 4.60 Performing valve exchange

2) Follow the screen prompts.

Note: For further details refer to the 'Maintenance' section of this manual.

5 Materials required

This chapter describes the consumables and accessories required for the operation of your autoMACS Pro Separator.

IMPORTANT: Please be advised that the autoMACS Pro Separator is specified for use with MACS MicroBeads, autoMACS Columns, and other genuine Miltenyi Biotec consumables and accessories only. Please only use consumables and accessories recommended by Miltenyi Biotec. Failure to use recommended consumables and accessories may result in inaccurate results, device malfunction or damage, premature wear and reduced life time of the instrument. Miltenyi Biotec does not honor any warranty or accept any responsibility for damages resulting from the use of inappropriate consumables or accessories.

5.1 Materials required for operation

5.1.1 Solutions

For daily operation, the following solutions are required: Running Buffer, Washing Solution, and Ethanol.

Solution	Description	Color code	Capacity	Order no.
Running Buffer	autoMACS Running Buffer or MACS Separation Buffer	Blue	6 × 1.5 L for 6 × 15 separation and rinsing cycles	130-091-221
Washing Solution	autoMACS Pro Washing Solution	Green	6 × 1.5 L for 6 × 15 rinsing cycles (Rinse, Sleep, Store, Safe)	130-092-987
Storage Solution	70% v/v ethanol in distilled water (prepared from absolute Ethanol)	Black		Not available

Table 5.1 Solutions required for daily operation

Solution bottles can be identified by color code and symbols (Table 3.1 Symbols and color coding of fluid containers).
For proper operation of the autoMACS Pro Separator, solution containers must be filled with a minimum volume of 150–200 mL. It is recommended to use ready-to-use MACS

Buffers or fresh, filter-sterilized solutions to prevent potential contamination of the tubing system.

> **Note:** MACS Running Buffer contains azide preservative. A solution without preservative may be prepared by diluting MACS BSA Stock Solution (130-091-376) 1:20 with autoMACS Rinsing Solution (130-091-222)

The autoMACS Pro Washing Solution is a filter-sterilized and ready-to-use solution to rinse the fluidic system after any autoMACS Pro Cell Separation. It contains a detergent that dissolves cell aggregates. The autoMACS Pro Washing Solution was developed for optimal cleaning of the autoMACS Pro Tubing System.

70% ethanol

This solvent should be prepared by diluting absolute ethanol with distilled water.

> **Note:** Do not use denatured ethanol (technical ethanol), as the autoMACS Columns are not resistant to oxidative compounds. Use 100% ethanol, analytical reagent grade, without additive.

5.1.2 Hardware

MACS MiniSampler

The MACS Mini Sampler can be equipped with three different tube racks (for details, see table below). To attach the MiniSampler and the tube racks, see section 3.2.3. The autoMACS Pro Separator will automatically detect the MiniSampler and the type of tube rack.

Tube racks, tubes and reagent racks

Three tube racks, Chill 5, Chill 15, and Chill 50, designed for 5 mL, 15 mL, and 50 mL tubes, respectively, and are available for use with the autoMACS Pro Separator. All three racks contain a coolant. To cool the racks, keep them in a refrigerator for 3–4 h. Do not cool below 0 °C to avoid freezing of the samples. Use 5 mL, 15 mL, or 50 mL tubes. The process has been optimized using BD Falcon™ tubes.

Rack type	Tubes	Maximum number of samples	Maximum sample volume	Maximum number of cells per sample
Chill 5	5 mL	6	2.5 mL	5×10^8
Chill 15	15 mL	5	12.5 mL	2.5×10^8
Chill 50	50 mL	3	Up to 50 mL	4×10^8
Reagent Rack 4	4 vials	Not applicable	Not applicable	Not applicable

Table 5.2 Cooling tube and reagent racks.

5.2 Materials required for maintenance

5.2.1 Solutions

MACS Bleach Solution

MACS Bleach solution (130-093-663) is used in combination with the **Safe** program. Depending on the level of use, it is recommended to run a **Safe** program at least every 3 to 6 months. If material like Whole Blood or tissue is primarily used it is recommended to run program **Safe** once a month.

> **Note:** Program **Safe** can also be used for decontaminating the autoMACS Pro fluidic system. For special decontamination procedures contact the technical support team for further advice.

Disinfectant solution

It is recommended to clean the ports of the automated arm and the surface of the instrument of spilled fluid with 70% ethanol or isopropyl alcohol on damp tissue. Alternatively, use alcohol swabs.

Distilled water

It is recommended to use distilled water on tissue to remove any salt crusts from the instrument.

5.2.2 Hardware

autoMACS Columns

Order no. 130-021-101 (5 × 2 columns)
Capacity of 2×10^{8} magnetically labeled cells from up to 4×10^{9} total cells.

Column substitutes

Order no. 130-090-835 (2 pieces)
For installation prior to storage of the autoMACS Pro Separator for longer than two weeks. The instrument is delivered with installed column substitutes.

Hydrophobic 0.2 µm air filters

Order no. 130-090-385
Hydrophobic air filters are used to vent fluid containers and to prevent aerosols. Do not use hydrophilic filters, since they are easily blocked upon contact with liquid and thus may cause errors.

autoMACS Pump Syringe

Order no. 130-090-339
(1-2 required per year)

Wrench

Order no. 130-090-378

For tightening tube connections. Included in the autoMACS Pro Starting Kit.

autoMACS Pro Laminar Hood Plate

Order no. 130-093-246

The metal plate has been developed for stable placement of the autoMACS Pro Separator on potentially bending surfaces; for example, the surface in a laminar flow hood or safety cabinet. The thick metal plate is designed to stabilize the instrument and maintain optimal alignment with the MACS MiniSampler.

autoMACS Pro Angle Connector Set

Order no. 130-093-245

The autoMACS Pro Angle Connector Set has been designed for placement of the autoMACS Pro Separator in a location with limited space. Cables that connect the back of the instrument, i.e., the fluid sensor cable, the MACS MiniSampler cable, and the 2D code reader cable are connected at a 90° angle to reduce the total depth of the instrument. The set contains three Sub-D-pin adaptors.

6 Cell separation using the autoMACS™ Pro Separator

The following chapter describes how to perform cell separations using the autoMACS Pro Separator.

IMPORTANT: The autoMACS Pro Separator is intended for research applications only and not for diagnostic or therapeutic use.

The procedure of cell separation has been categorized into seven steps.

1. **Prepare cell samples**: A single-cell suspension devoid of dead cells, aggregates, and cell debris is the prerequisite for efficient cell separation.
2. **Select a cell separation strategy and a suitable magnetic labeling strategy**: Prior to cell separation samples are labeled by magnetic MicroBeads. This can be performed manually or automatically using the autoMACS Pro Separator. Cell separation strategies are classified as:
    - Positive selection or depletion.
    - Direct or indirect magnetic labeling.
    - Cell sorting based on one or more marker(s).
3. **Determine number of samples**: Select an appropriate tube rack for the number of samples and also take the sample volume into account.
4. **Prime the autoMACS Pro Separator**: Before performing a cell separation, the autoMACS Columns must be filled with Running Buffer by running the **Rinse** program.
5. **Define autoMACS Pro Separation Programs on the sample rack template**: Select a separation and a washing program for every cell sample or, labeling separation wash program

    Separation programs
    - Positive selection or depletion
    - Standard mode or sensitive mode
    - Single or double column selection
    - Special programs for efficient depletion of unwanted cells or cells that are only dimly labeled
    - Special programs for cell separation directly from whole blood

    Washing programs
    - Quick Rinse (**Qrinse**) or **Rinse** are washing steps between separations
    - **Sleep**—to follow the last program before the instrument is switched off
6. **Run the autoMACS Pro Cell Separation**: The autoMACS Pro Separator automatically isolates target cells from up to six independent samples in a sensor-controlled manner.

7. **Shutdown the autoMACS Pro Separator**: A **Sleep** or **Store** program for overnight storage or long-term storage, respectively, and must be performed before shutting down the autoMACS Pro Separator.

6.1 Prepare cell samples

In order to obtain optimal separation results, some crucial points must be considered:

6.1.1 Prepare single-cell suspensions

Cell aggregates may contain mixtures of target and non-target cells and therefore can impair the separation results. To minimize the risk of cell aggregation MACS Separation Buffer/MACS Running Buffer should be used during sample handling steps. Resuspend cells carefully after centrifugation. For specific recommendations, please refer to the general protocol section of the user manual or to the respective MACS Cell Separation Reagent data sheet.

> **Note:** Use pre-separation filters when cell clumping is suspected, especially when working with previously frozen material, cord blood, dissociated tissue, or whole blood. Cell pre-filtration removes aggregates, dead cells, and debris.

Large cell aggregates may interfere with the separation process and may cause pressure variations in the autoMACS Pro fluidic system. It is recommended to use Pre-Separation Filters (# 130-041-407) to remove cell clumps that may clog the column.

Dead cells and cell debris may bind non-specifically to MACS MicroBeads, antibodies, and antibody conjugates. To remove dead cells, it is recommended to use density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).
For specific recommendations, please refer to the general protocols in the corresponding section of this user manual or to the respective MACS Cell Separation Reagent data sheet.

6.1.2 Reagent volumes and labeling volumes must be adjusted to the total cell number

Typically, 10^7 cells are labeled in a total volume of 100 µL. When working with higher cell numbers, scale-up all reagent volumes and total volumes accordingly. For example, for 2×10^7 total cells use twice the volume of all indicated reagent volumes and total volumes indicated in the respective data sheet. When working with fewer than 10^7 cells, do NOT scale down the volume. For specific recommendations, please refer to the respective MACS Cell Separation Reagent datasheet.

For details concerning diluent volumes required for the first labeling step refer to Table 6.1 below.

MACS Product	Strategy	Reagents	Cell concentration	Minimal volume*
Direct MicroBeads – human – rat – non-human primate	Positive selection or Depletion	1	10^7 cells per 80 µL	160 µL
Direct MicroBeads – mouse	Positive selection or Depletion	1	10^7 cells per 90 µL	180 µL
Whole Blood MicroBeads (Chill 50)	Whole blood or bone marrow	1	Original volume	4 mL – 8 mL
Cell Isolation Kits	Untouched selection	2	10^7 cells per 40 µL	160 µL
Cell Isolation Kits	Untouched selection	3	10^7 cells per 30 µL	120 µL
MicroBead Kits	Positive selection or Depletion	2	10^7 cells per 60 µL	120 µL
* When working with fewer cells than the necessary minimal volume, resuspend cells in the stipulated minimal volume.				

Table 6.1 Table shows dilution volumes required for the first autolabeling step.

6.2 Select a cell separation strategy and a suitable magnetic labeling strategy

The autoMACS Pro Separator can perform fully automated sample processing, i.e., sample autolabeling, labeled cell incubation, cell separation and target cell elution. The software also offers the choice to perform cell separation on manually labeled cells. Thus, it is possible to perform automated cell separation with autolabeling; automated cell separation without autolabeling; or, automated cell separation using a combination of both strategies.

6.2.1 Choosing a cell separation strategy

There are two basic strategies for separating specific cell populations: positive selection and depletion. Using the positive selection strategy, the target cells are magnetically labeled and collected as the positive fraction. Using the depletion

strategy, the unwanted cells are labeled, separated from the target cells, and eluted as a positive cell fraction i.e., cells labeled with MicroBeads. The target cells will be collected in the negative fraction. Both strategies can be combined for multiparameter sorting.

Positive selection

Positive selection allows up to 10,000-fold enrichment of the magnetically labeled target cells. Positive selection takes advantage of the high specificity of monoclonal antibodies to isolate highly pure cell populations that specifically express the corresponding antigen. The positively selected cells are virtually unaffected by the separation procedure and can be used immediately for culturing or other applications. There is no need to remove the MACS MicroBeads from the cells. The small size and the composition of the MACS MicroBeads (iron oxide and polysaccharide) make them biodegradable. Typically, MACS MicroBeads do not activate cells or influence function or viability.

Positive selection is recommended:

- for highly specific labeling
- for excellent purity and recovery
- for the isolation of rare cells
- if no subsequent separation step is needed

Depletion

For some experiments it may be desirable to deplete certain cell types from the cell sample in order to isolate the target cells. The depletion strategy allows, for example, to isolate a target cell for which no specific antibody is available or to isolate “untouched” cells.
With a depletion strategy all unwanted cells are magnetically labeled. During magnetic separation, the labeled cells are retained on the column, while the target cells pass through and form the negative, non-labeled fraction.

A depletion strategy is recommended:

- for the removal of unwanted (non-target) cells
- if no specific antibody is available for the target cells
- if binding of minimal amounts of antibody to surface molecules can interfere with downstream applications
- if isolated cells are to be sorted subsequently utilizing a second marker

Multiparameter sorting : depletion followed by positive selection

Cells can also be isolated by first depleting the non-target cells, followed by magnetic labeling and positive selection of the target cells. This strategy is useful if unwanted cells in the cell suspension express the same antigen that was chosen as the marker for positive selection.

It can also be used to deplete non-target cells from the initial cell suspension before isolating extremely rare cell types by positive selection.

MACS MultiSort strategy

With MACS MultiSort Kits, high numbers of cells characterized by multiple cell surface markers can be sorted easily and quickly. Even rare cells can be enriched efficiently. Multiparameter sorting with MACS MultiSort Kits allows sequential positive selections of cells. The target cells are first labeled with MACS MultiSort MicroBeads and positively selected for the first parameter. Subsequently, the cells are incubated with the MultiSort Release Reagent, which enzymatically removes the MicroBeads from the antibodies. In the next step, these cells are magnetically labeled with MicroBeads conjugated with an antibody directed against a second marker. After labeling, the cells are again magnetically separated.

Magnetic labeling strategies

There are two basic approaches to magnetic labeling: direct labeling with MACS MicroBeads and indirect labeling with MACS MicroBeads against primary antibodies, other specific ligands or their conjugates. Both approaches can also be used for a MACS MultiSort strategy.

Direct magnetic labeling

Direct magnetic labeling is the fastest way of magnetic labeling. It requires only one labeling step as the specific antibody is directly coupled to the magnetic particle. Direct labeling minimizes the number of washing steps and, therefore, avoids cell loss. Highly specific monoclonal antibodies have been selected by Miltenyi Biotec to produce a large variety of antibody-conjugated MicroBeads targeting many human, mouse, rat, and non-human primate cell surface markers. The high specificity of the antibodies allows low background and easy optimization of the separation. Fluorescent staining using fluorochrome-conjugated antibodies can simultaneously be performed for subsequent analysis of the separated fractions by flow cytometry or fluorescence microscopy.

Indirect magnetic labeling

Indirect labeling is recommended when no direct MicroBeads for a particular cell surface marker are available. Almost any monoclonal or polyclonal antibody or other specific ligand targeting any cell type from any species can be used for indirect labeling. Cells are first incubated with a primary antibody ligand that is unconjugated, biotinylated, or fluorochrome-conjugated. In a second step, magnetic labeling is performed by using Anti-Immunoglobulin, Anti-Biotin, Streptavidin, or Anti-Fluorochrome MicroBeads, respectively.

A cocktail of antibodies or other ligands can also be used to concurrently isolate or deplete a number of cell types. As it results in an amplification of the magnetic label, indirect labeling may be the method of choice if weakly expressed markers are targeted for magnetic separation. When using a fluorochrome-conjugated antibody or other ligand in combination with the corresponding Anti-Fluorochrome MicroBeads, the fluorescent staining can be used for flow cytometric analysis.

Note: Titration and manual incubation with the primary antibody or other ligand is recommended to be done manually. If there is no wash step between incubation of the sample with an unknown primary antibody or other ligand followed by application of indirect MicroBeads, the procedure cannot be optimized. Results may be impaired.

Cell isolation directly from whole blood or bone marrow samples

MACS Whole Blood MicroBeads are specially developed to isolate human cell subsets directly from human whole blood using the autoMACS Separator or the autoMACS Pro Separator. This reagent can also be applied to bone marrow. Note that in this case fat must be removed prior to labeling.

6.2.2 Choose a magnetic labeling strategy

Autolabeling may be performed prior to cell separation. Autolabeling is optimized for the following MACS Products:

- Products consisting solely of a direct MicroBead labeling strategy when the target cell frequency is >5%. FCR-Blocking Reagent can also be used for direct MicroBead labeling.
- Products consisting of a single Biotin conjugate or a Biotin Cocktail with Anti-Biotin MicroBeads, where no washing step is required between both labeling steps. See the relevant datasheet for more information.

Autolabeling at a glance

1) Assign MicroBead reagent vials to positions on the MACS Reagent Rack.
It is recommended to use the 2D code reader. Alternatively, the product order number may be manually programmed. See section 4.1.2 for full details.

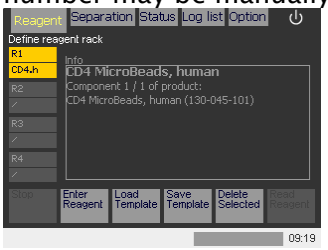
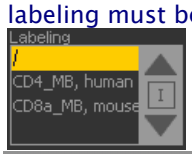


Figure 6.1 'CD4 MicroBeads, human' vial was placed onto the MACS Reagent Rack position 'R1'.

2) On the **Separation** tab the recommended separation strategy and wash program are pre-selected for the chosen autolabeling reagents.

3) Program the cell separation protocol using the **Separation** tab menu. For full details see section 4.1.3

Note: '/' option under **Labeling** denotes that NO autolabeling will be performed. Only a cell separation will be performed; manual magnetic labeling must be performed prior to placing the sample on the Chill Rack.



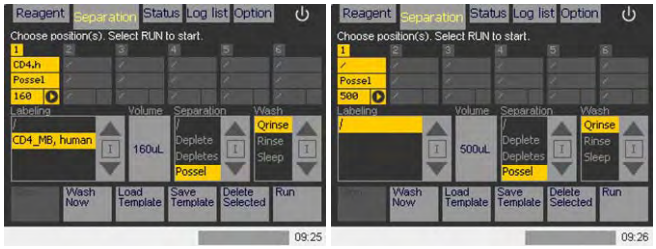


Figure 6.2 Performing MicroBead CD4+ cell separation with autolabeling (left) and without autolabeling (right). The cell separation (Possel) and wash conditions (Qrinse) for both processes are identical. Disabling autolabeling ('/') influences the initial sample volume. For manual labeling it is recommended to dilute cells to a volume of 500 µl / 10^8 total cells (see corresponding datasheet for further information).

> **Note:** Refer to the product datasheet for more detailing on sample magnetic labeling volumes.

4) Select **Run**.

Manual labeling at a glance

Cells are labeled with MACS MicroBeads according to the MACS Cell Separation Reagent data sheet. For general immunomagnetic cell separation protocols, please see section 8.1. The specific MACS Cell Separation Reagent data sheets can be found at www.miltenyibiotec.com.

6.2.3 autoMACS™ Pro Cell Separation programs

The autoMACS Pro Cell Separation programs are based on different cell separation strategies. There are ten preset separation programs to choose from depending on the cell isolation strategy, the frequency of target cells, and the level of antigen expression.

Positive selection programs:

Possel—Positive selection in standard mode: isolation of cells with normal antigen expression and frequencies higher than 5%; select Possel if purity is the highest priority.

Possel_s—Positive selection in sensitive mode: isolation of cells with low antigen expression and frequencies higher than 5%; select Possel_s if yield is the highest priority.

Posseld—Positive selection in standard mode I, double-column program: for isolation of rare cells in low elution volume.

Posselds—Positive selection in sensitive mode, double-column program: for isolation of rare cells with low antigen expression.

Posseld2—Positive selection in standard mode II, double-column program: for isolation of rare cells.

Posselwb—Special positive selection in special mode, double-column program: for isolation of cell subsets from whole blood. Cell samples are automatically diluted with Running Buffer.

Depletion programs:
Deplete—Depletion in standard mode: for removal of cells with normal to high antigen expression and results in better target cell yield.
Depletes—Depletion in sensitive mode I: Removal of cells with low antigen expression and results in better target cell purity.
Depl05—Depletion in sensitive mode II: Removal of cells with low antigen expression and results in stringent depletion of cells.
Depl025—Depletion in sensitive mode III: Removal of cells with low antigen expression and results in stringent depletion of cells.

Positive selection programs
In programs **Possel and Possels**, the magnetically labeled target cells are retained in the autoMACS Column 1. The unlabeled cells are released into the negative fraction collection tube (row "B" of the tube rack). After automated retraction of the magnet, the magnetically labeled cells are eluted into the positive fraction collection tube (row "C" of the tube rack).
In the double-positive selection programs **Posseld, Posselds, Posseld2, and Posselwb**, the magnetically labeled target cells are first retained in the autoMACS Column 1. The negative fraction containing the non-labeled cells is retrieved in the negative fraction collection tube (row "B" of the tube rack). Then, the magnetically labeled cells are held in a reservoir and loaded onto the autoMACS Column 2. Finally, the magnetically labeled cells are eluted into the positive fraction collection tube (row "C" of the tube rack).

Depletion programs
When running any **depletion** program, the magnetically labeled non-target cells are retained in the autoMACS Column 1. The non-labeled target cells pass through the column and are released into the negative fraction collection tube (row "B" of the tube rack). The magnetically labeled fraction, containing the unwanted cells, is eluted into the positive fraction collection tube (row "C" of the tube rack).

Program	Volume of non-labeled fraction (i.e. negative fraction)	Volume of labeled fraction (i.e. positive fraction)	Loading rate
Deplete	2 mL + sample volume	2 mL	4 mL/min
Depletes	2 mL + sample volume	2 mL	1 mL/min
Depl05	2 mL + sample volume	2 mL	0.5 mL/min
Depl025	2 mL + sample volume	2 mL	0.25 mL/min
Possel	2 mL + sample volume	2 mL	4 mL/min
Possel_s	2 mL + sample volume	2 mL	1 mL/min
Posseld	2 mL + sample volume	0.5 mL	4 mL/min (column 1) 1 mL/min (column 2)
Posseld2	2 mL + sample volume	2 mL	4 mL/min (columns 1 and 2)
Posselds	2 mL + sample volume	2 mL	1 mL/min (columns 1 and 2)
Posselwb	2 mL + sample volume + predilution volume	2 mL	4 mL/min (columns 1 and 2)

Table 6.2 Output volumes and loading rates of separation programs.

6.2.4 autoMACS™ Pro Separator wash programs and maintenance programs

Wash programs for daily operation

The autoMACS Pro Separator is equipped with reusable autoMACS Columns. After each cell separation, a thorough washing procedure rinses the columns of the autoMACS Pro Separator. After the wash program is completed, columns and tubing system are filled with Running Buffer.

Program	Washing Solution	Running Buffer	70% Storage Solution	MACS Bleach	Time
Qrinse	---	48 mL	---	---	1.5 min
Rinse	96 mL	48 mL	---	---	4 min
Sleep	96 mL	---	48 mL	---	5 min
Store	96 mL	---	96 mL	---	8 min*
Col_ex	96 mL	96 mL	---	---	6 min*
Safe	96 mL	96 mL	---	40 mL	21 min*§

Table 6.3 Liquid usage and time of wash and maintenance programs.

*** Not including the time required for column exchange**

§ Not including the time required for disconnecting and reconnecting bottle tubing

Note: The autoMACS Pro Separator will not start a separation program before a wash program has been completed.

Qrinse is the standard short wash program that only uses Running Buffer. It is recommended to use this program between separations of cells with normal frequency.

Rinse is an extensive rinsing program that uses Washing Solution and Running Buffer. It is recommended to use this program between separations of rare cells, e.g. stem cells, the separation of cells from different species, and is mandatory between whole blood separations.

Sleep—it is highly recommended to use **Sleep** as the last wash program before overnight storage. Upon completion of the **Sleep** program, the fluidic system contains 70% ethanol.

Maintenance programs

The program **Safe** is designed for decontamination of the fluidic system. For details, see section 7. Upon completion of the **Safe** program, the fluidic system contains Running Buffer. The program includes an exchange of autoMACS columns.

The program **Store** should be applied to prepare the instrument for long-term storage. Upon completion of the **Store** program, the fluidic system contains 70% ethanol. For details, see section 7.

The program **Col_ex** is used for column exchange. Upon completion of the **Col_ex** program, the fluidic system contains Running Buffer.

6.3 Select appropriate tube rack

Three different tube racks are available for processing sample volumes between 0.2 mL and 50 mL. Unless otherwise specifically indicated in the MACS Cell Separation Reagent data sheet, the magnetically labeled cell samples are suspended at 10^8 total cells per 500 µL.

1) Select the appropriate tube rack according to Table 6.4.
2) (Recommended) Cool down the tube rack for 3–4 hours in a refrigerator (2–8 °C) or until the coolant becomes solid. **Do not cool below 0 °C as samples may freeze.**
3) Equip the tube rack with sample tubes and fraction collection tubes.

Note: Row “A” of the tube rack holds sample tubes; row “B”: tubes for non-labeled fractions; row “C”: tubes for labeled fractions.

Rack type	Slots	Maximum number of samples	Minimum first incubation volume	Maximum final labeling volume	Maximum number of cells per tube*
Chill 5	24 × 5 mL	6 (5 mL tubes)	0.2mL	2.5 mL	2.5–5.0x10E8 depends on cell labeling concentration and column capacity
Chill 15	15 × 15 mL 5 × 5 mL	5 (15 mL tubes)	0.2mL	6.5 mL	6.5x10E8–1.3x10E9 depends on cell labeling concentration and column capacity
Chill 50	6 × 50 mL 3 × 15 mL 3 × 5 mL	3 (50 mL tubes)	4 mL	8 mL	Only for whole blood
* Values apply to PBMC preparations. These are autolabeling volumes.					

Table 6.4 MACS Cooling Tube Racks: Chill Racks 5, 15 and 50

6.4 Prime the autoMACS™ Pro Separator

Note: It is assumed that the MACS MiniSampler has been already installed, the waste container is empty, and that all fluid containers are filled with recommended solutions (see section 3 for more details).

1) Switch on the autoMACS Pro Separator and wait for the instrument to complete initialization.

Figure 6.3 The initialization screen.

2) After initialization, the autoMAC Pro Separator will display the menu **Status**. Verify the status of the instrument as outlined in section 4.1.4.

3) Ensure that the symbols for the fluid containers are colored green. If they are red, check whether the fluid containers are filled, the waste container is empty, and the color-coded bottle sensors are connected to the appropriate container. If symbols are gray, check the bottle sensors for proper connection.

Figure 6.4 Fluid container status symbols. Left: Fluid containers are ready. Right: Fluid containers need replaced.

> **Note:** 70% ethanol does not contain electrolytes. Therefore, the fluid level cannot be determined and the symbol for this bottle is always gray. Please ensure that the volume of each solution is sufficient for the number of separation and washing programs to be performed.

4) Ensure that the symbol for the columns is colored green. For more details see section 4.1.4: Column status. If the symbol for the columns is red, the columns must be exchanged. The fill level on the symbol is an indicator for the remaining service-life of the columns.

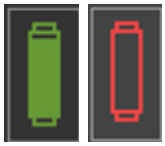


Figure 6.5 The column status graphic.
Left: The columns are ready. Right: The columns must be changed.

> **Note:** The autoMACS Pro Separator automatically records the date of the last column exchange and displays the date for column replacement only if the program **Column exchange** has been used for column installation.

5) Ensure that the MACS MiniSampler is installed correctly. For more details see section 4.1.4: MACS MiniSampler status.

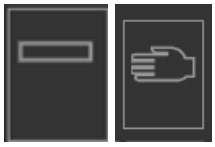


Figure 6.6 MACS MiniSampler status graphic.
Left: The MiniSampler was successfully installed. Right: No MiniSampler was detected.

> **Note:**The touchscreen displays the type of tube rack upon starting the separation.

6) The autoMACS Pro Separator is now ready for priming. Select **Separation,** and **Wash now** from the lower navigation bar.

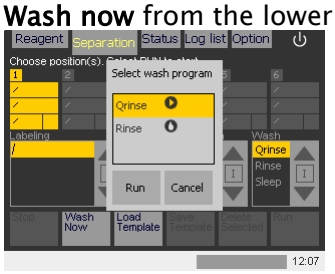


Figure 6.7 'Wash now' command displays two options: 'Qrinse' (quick rinse) and 'Rinse'. A full rinse is required for instrument priming.

7) Select **Rinse** and **Run**.

> **Note:** If priming of the instrument has not been performed, the autoMACS Pro Separator will automatically ask to run a **Rinse** program before starting the separation. The process can be monitored in the menu **Status**. At this point, the bottles are illuminated yellow.
>
> **Note:** It is possible to include an initial **Rinse** program that will be performed automatically upon switching on the instrument. The setting of this option is described in section 4.1.6. O_init: Optional priming of the instrument at startup.

6.5 Define autoMACS™ Pro Separation program sequence or template

After completing the priming process or if a wash program has been completed, the autoMACS Pro Separator is ready for separation. The status of the instrument is displayed in the menu **Status**. The bottles are illuminated green. Select the **Separation** menu.

> **Note:** See sections 4.1.2 and 4.1.3 for an overview of the **Reagent** and **Separation** tabbed menus, respectively.

1) Select the desired position(s) in the sample separation template field by touching it.

2) Select an autolabeling program and the sample volume (if needed), a separation program, and a washing program for each sample position. The selected programs will be displayed in the programming field.

> **Note:** For autolabeling the Reagent Rack configuration must be programmed using the **Reagent** tab menu. See section 4.1.2 or 6.2.2 section for further details.
>
> **Note:** To obtain brief information on the separation and wash programs, highlight the program of interest and press the information button (I)

located between the arrows. A pop-up window will be displayed. Alternatively, refer to section 6.2.3 for more information on separation programs.

3) (Optional) Templates can be saved by selecting **Save template** from the lower navigation bar. Follow the prompt to enter a template name.

4) Select **Ok** and **Run**.

To select a particular sample position in the programming field, touch it once. To deselect it, touch it once again.

If the same combination of separation program and washing program is required for more than one sample, highlight all the desired sample positions first and then select the programs. Alternatively, define the program combination for one position and then highlight the other desired positions to adopt the program combination.

To erase selected program combinations from the programming field, first mark the respective positions and choose **Delete selected** from the lower navigation bar. If positions are not marked, the button can be used to delete the entire template. In this case, the option Delete selected will switch to **Delete all**.

Alternatively, start the separation from a previously saved separation template.

1) Select **Load template** from the lower navigation bar.

2) Select the desired template by using the arrows.

3) Select **OK**.

4) Select **Run** to start the separation. Check that there is enough buffer for the number of programmed separations.

5) Select **Continue**.

The autoMACS Pro Separator automatically detects the type of tube rack in use and allows the user to utilize only the number of samples and sample positions the tube rack can handle. If the tube rack does not match the template definition, a warning screen will be displayed upon starting the separation.

6.5.1 Entering sample separation instructions: a workthrough example

In the following example two samples were placed on positions 1 and 2 of a Chill Rack 15. Sample 1 was already manually labeled using CD4 MicroBeads, human. Sample 2 has not yet been labeled; the autoMACS Pro Separator will perform autolabeling using CD4 MicroBeads, human.

Note: The MACS Reagent "CD4 MicroBeads, human" has been identified by the 2D code reader (barcode reader). The vial was placed on position 1 of the MACS Reagent Rack. Refer to section 4.1.2 for full details on using the **Reagent** menu.

1) Highlight sample positions 1 and 2 on the sample template.

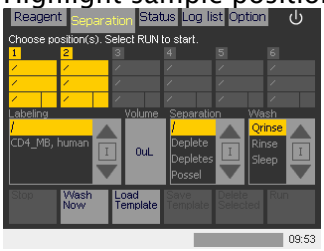


Figure 6.8 Sample positions 1 and 2 were selected – the purpose of this example is to demonstrate how sample processing conditions can be easily modified and to clarify the differences between autolabeling and manual labeling. Alternatively, each sample could be programmed individually.

2) Select **CD4_MB.human** under the submenu **Labeling**.
By default the software selects a **Possel** (positive cell/target cell enrichment) separation strategy and a **Qrinse** (quick rinse) wash protocol.

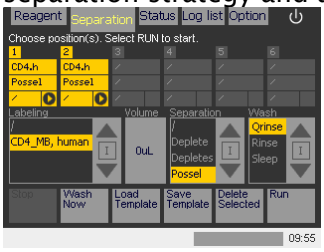


Figure 6.9 The current display indicates that autolabeling will be performed on both samples.

3) Select the **Volume** submenu to enter the required sample volume. Select **Enter**. For this experiment a minimal starting volume of 160 µL is required for autolabeling (for CD4 autolabeling 40 µl MicroBeads is added to the entire 160 µl sample volume). Refer to the data sheet if this information is unknown.

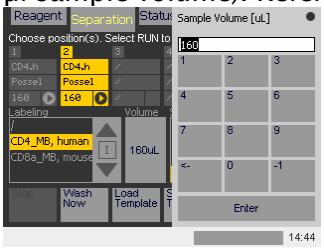


Figure 6.10 A volume of 160 µL was entered for both sample positions.

Note: Both samples positions have been setup for autolabeling.

4) Highlight the wash mode to be performed before cell separation. In this case a quick rinse (**Qrinse;**) was selected.

Note: Sleep, , can only selected as the last step in the program sequence.

5) Deselect sample position 2 by touching the display at this position.
Sample position 1 must be setup to perform cell separation on a sample that was previously manually labeled with CD4 MicroBeads.

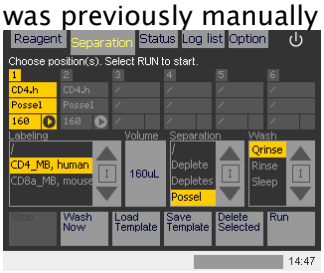


Figure 6.11 Only sample position 1 is selected.

6) Deselect autolabeling for sample 1 by selecting '/' in the **Labeling** submenu. Change the sample volume as recommended by the datasheet. For manual labeling it is recommended to dilute cells to a volume of 500 µl / 10^8 total cells (see corresponding datasheet for further information).

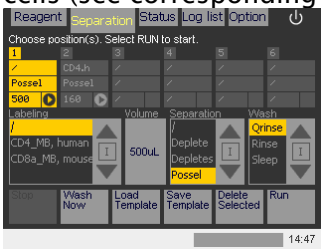


Figure 6.12 Only a positive cell separation will be performed on sample 1.

7) View and recheck the sample setup.

8) Select **Run** to start.

9) Check buffer levels as instructed by a popup dialog box. Click **Continue** to proceed.

10) It is recommended to monitor the process from the **Status** menu. Click the **Status** menu.

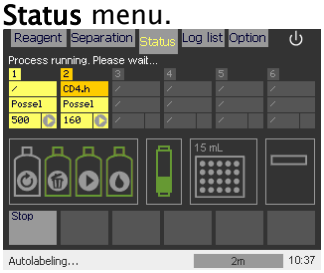


Figure 6.13 Sample processing is underway as instructed.

Note: Refer to section 4.1.4 for more information for clarification of the **Status** menu.

6.6 Monitoring the autoMACS™ Pro Separator during cell separation

The autoMACS Pro Separator is a sensor-controlled device that allows easy monitoring during operation.

6.6.1 Status menu before separation

Refer to section 4.1.4 for an overview of how to monitor the instrument status using the **Status** menu prior to performing a cell separation. Nevertheless, a brief summary is included below.

Status of fluid containers

The status of fluid containers is indicated by color-coded graphic symbols and by a text table.

Container	Symbol	Symbol color and user action
Running Buffer		Green: No action required Red: Refill container Gray: Connect bottle sensor
Washing Solution		
Storage Solution		Gray: No liquid detection; visually check volume
Waste		Green: No action required Red: Empty waste Gray: Connect bottle sensor

Table 6.5 Status of fluid containers displayed in the 'Status' menu

Column status

If the column symbol is green, no action is required. If the symbol is red, the columns must be exchanged. The level of the green fill on the column symbol indicates the remaining service-life of the autoMACS Columns.

Rack detection

Rack detection only occurs upon starting the separation process.

MACS MiniSampler detection

If the MACS MiniSampler has been detected correctly a rectangular symbol is displayed. If it has not been detected a hand symbol will be displayed in the same field.

6.6.2 Status menu during cell separation

It is recommended to monitor the instrument's status during cell separation using the **Status** menu. Programs yet to be processed appear in yellow fields. Programs currently undergoing autolabeling appear in lilac. Programs in progress in which no autolabeling is being performed switch to orange; completed programs switch to white. The current action is always displayed in the status bar located below the lower navigation bar. The status bar also displays the overall progress in minutes (min).

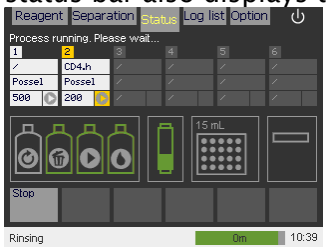


Figure 6.14 Monitoring the status during cell separation. Sample processing at position 1 is completed (white color). Sample at position 2 has finished the separation and is now performing a Rinse.

Interrupting cell separation

The cell separation process can be paused at any time from all menu screens by selecting **Stop**.

1) From any menu screen, select **Stop**. The autoMACS Pro Separator will then immediately stop operation and will display a pop-up warning screen from which the current program can be continued or canceled.

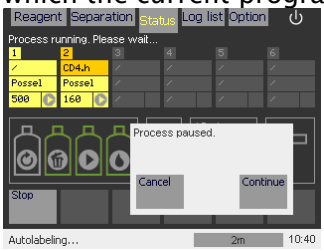


Figure 6.15 Select 'Stop' to pause cell separation. To continue cell separation select 'Continue'. To cancel the entire procedure, select 'Cancel'.

2) Select **Cancel** to cancel the procedure. Alternatively, select **Continue** to carry on with cell separation.

Figure 6.16 The process has been cancelled.

> **Note:** Interrupting the process after labeling will result prolong the incubation period.

Bottle illumination

The autoMACS Pro Separator has a bottle illumination that facilitates monitoring of the instrument's status. The table below summarizes the color code of the bottle illumination and the respective user action required.

Code	Status	User action
Green	Ready for separation	No action required
Blue	Instrument operating	No action required
Yellow	Not ready for separation	Run wash program (**Rinse** or **Qrinse**) before starting a separation
Red	Error	Check screen for error detection
Purple	Program **Sleep** is completed	Switch off autoMACS Pro Separator
Blinking	Action required	Check screen for required action

Table 6.6 Various bottle illumination statuses.

The bottle illumination can be switched ON/OFF.

1) Select the **Option** menu and **User settings** by touching.
2) Highlight **O_led** and press **Run**. The bottle illumination can now be enabled or disabled.

6.7 Shut down the autoMACS™ Pro Separator

6.7.1 Sleep as the final wash program

1) Combine a separation program and the program **Sleep** for the last position in the programming field.
2) Upon completion of the **Sleep** program, switch off the autoMACS Pro Separator using the main power switch.

Note: If **Sleep** is chosen as a wash program, the autoMACS Pro Separator will not allow definition of any programs beyond this position.

Note: The autoMACS Pro Separator automatically performs a **Sleep** program if the device is inactive for more than 6 hours.

Note: Ensure that enough Storage Solution is in the bottle.

6.7.2 Store: the program for long-term storage

To store the autoMACS Pro Separator for a period longer than two weeks, the tubing system should be cleaned and the columns should be replaced with column substitutes. The **Store** program automatically performs the cleaning procedure and prompts the user to install column substitutes.

1) Select **Option** and **Special**.
2) Select **Store** and press **Run**. The system will be rinsed automatically.

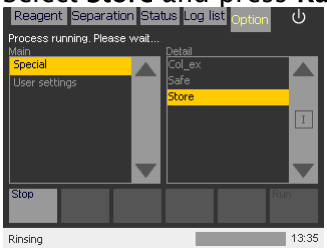


Figure 6.17 Running the 'Store' program.

3) Install the column substitutes (see section 3.2.8 for details on column exchange).
4) Select **Done**.
5) Switch off the autoMACS Pro Separator using the main power switch.

6.7.3 Shutdown button

1) Press the shutdown button on the upper right hand corner of the screen ( ).
2) Select **Yes** from the popup dialog box.

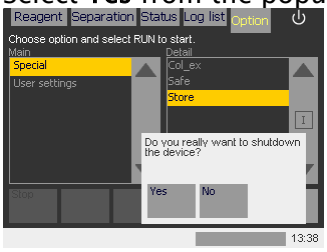


Figure 6.18 Shutting down the autoMACS Pro Separator.

3) The autoMACS Pro Separator will automatically perform a **Sleep** program.
4) Upon completion of the **Sleep** program, you will be prompted to shut down the device. The bottle illumination is purple at this point. Switch off the autoMACS Pro Separator by using the main power switch. **Note:** Ensure enough storage solution is available.

7 Maintenance

Appropriate maintenance of the autoMACS Pro Separator helps to maintain excellent reproducibility of the cell separation results. The following section gives you an overview on the procedures required for efficient maintenance of the instrument.

IMPORTANT: Insufficient or improper maintenance of your autoMACS Pro Separator can cause unpredictable results, avoidable malfunction and premature failure of the instrument, and may void your warranty.

IMPORTANT: Please do not perform any maintenance procedures other than specifically described in this user manual. Any other maintenance procedures must be performed by qualified service personnel.

Inquire with your local Miltenyi Biotec representative about Miltenyi Biotec's extensive instrument service and support arrangements, or see www.miltenyibiotec.com/support.

IMPORTANT: When replacement or spare parts are required for maintenance, only use genuine Miltenyi Biotec parts or third-party parts specified and recommended by Miltenyi Biotec. Using unauthorized replacement or spare parts can cause malfunction of the device and impair cell separation results. Miltenyi Biotec does not honor any warranty or accept any responsibility for device failure or damages resulting from the use of inappropriate replacement or spare parts.

Caution: During a maintenance procedure, potentially contaminated liquid may spill out of the orifice of the washing station and the tubing. Therefore, wear protective gloves, protective clothing, and safety glasses to avoid contact with skin and eyes. Dispose used gloves and clothing appropriately.

7.1 Daily maintenance

1) Prime instrument: A **Rinse** program must be performed before performing the first cell separation after the instrument has been switched on. By using the **O_init** program, the instrument can be instructed to perform an automated rinse sequence at startup (see section 4.1.6). The **Rinse** program is used for efficient washing and equilibration of the fluidic system.

> **Note:** The instrument automatically prompts the user to perform a **Rinse** before performing a cell separation.

2) Clean uptake/outlet ports before each **Sleep** program. For details see section 7.13.

3) Instrument shutdown: Make sure to run a **Sleep** program prior to switching the instrument OFF; for details see section 6.7. This program ensures most efficient preservation of the fluidic system by rinsing with storage solution.

7.2 Periodic maintenance

1) Exchange autoMACS Columns every 2 weeks or after 100 separations, whichever comes first (see section 3.2.8).

2) Clean pump syringe every 1–3 months (see section 7.4.2).

3) Clean washing station: Remove spills and salt crusts as required (see section 7.11).

4) Run **Safe** program: For decontamination it is recommended to run the **Safe** program every 3 to 6 months (see section 4.1.6). More frequent running of the Safe program will not impair the instrument.

5) Long-term storage: Run the **Store** program and exchange the autoMACS Columns with substitute columns if you intend to store the instrument for a period longer than 2 weeks (see section 6.7.2).

7.3 Preventative maintenance

1) Exchange dilutor valve at least once per year* (see section 7.6).

2) Exchange valves at least once per year* (see section 7.5).

3) Exchange pump syringe at least once per year* (see section 7.4.2)

4) Exchange peristaltic pump head at least once per year (see section 7.12).

5) Exchange hydrophobic air filters at least once per year (see section 7.10).

***** Depending on the level of use and general instrument maintenance, however, these parts may need to be exchanged more frequently.

7.4 General considerations

Please consider that the sample quality has a significant influence on the system's performance. It is crucial to use single-cell suspensions for cell separation. Furthermore, dead cells and other small particles potentially derived from the sample preparation should be removed prior to cell separation. For further information on sample preparation, see section 6.1.

Note: Do not use a dishwasher to clean and do not autoclave any of the removable parts unless indicated otherwise.

7.4.1 Pump maintenance

The Pump Syringe requires periodic maintenance. It is recommended to clean the dilutor valve and the syringe every 1 to 3 months with distilled water. Removing salt deposits can prevent leakage of the fluidic system. The syringe should be replaced once a year. Depending on the level of use and general instrument maintenance, however, these parts might need to be exchanged more frequently.

7.4.2 Pump syringe exchange

1) Run a **Sleep** Program (see 6.7), switch OFF the power, and unplug the autoMACS Pro Separator from the electric supply. Open the front access cover. The syringe pump plunger should be at the top most position.
2) Loosen the plunger lock screw by turning it counter-clockwise.
3) Lower the plunger holder by firmly pressing the plunger lock screw downward.
4) Unscrew the syringe from the dilutor valve housing.

Note: To clean the syringe, carefully remove the plunger from the syringe. Remove salt crusts with distilled or deionized water. Use distilled or deionized water to wet the plunger and carefully push the plunger back into the syringe. Dry the plunger lock screw before proceeding with installation of the syringe.

5) To install the new syringe, carefully insert the syringe into the plunger holder.
6) Push the plunger holder up until the syringe reaches the dilutor valve.
7) Fasten the syringe at the dilutor valve by turning it until a resistance can be felt.
8) Tighten the plunger lock screw.
9) Prime the autoMACS Pro Separator as described in section 6.4.
10) Run the program **Calibr_2** to calibrate the fluidic volume control of the instrument. See section 3.2.7 for details.

Note: If symptoms of wear such as leakage persist, contact technical service.

7.5 Valve exchange

The fluidic system of the autoMACS Pro Separator is regulated by six valves. The valve that is connected with the pump syringe will be referred to as dilutor valve. Instructions for the exchange of the dilutor valve are provided in section 7.6.

If a valve of the autoMACS Pro Separator starts to leak, an exchange of the valve should be performed.

> **Note**: If wear of a valve is suspected, use the **Check_up** program to analyze functionality (for details, see section 4.1.6). If the dilutor valve requires exchange, refer to section 7.6.

Exchange one valve at a time according to the following instructions:

1) Select **User settings** from the **Options** menu. Highlight **Valve_ex** and press **Run** to start the program. Select valves which have to be exchanged. Valves will automatically be turned to exchange position.
2) Switch OFF and unplug the autoMACS Pro Separator. Open the front cover. For exchange of the lower valves, remove the bottom cover.
3) Remove all tubing and valve port locks from the respective valve.
4) Loosen the two valve screws using the screwdriver from the autoMACS Pro Separator Starting Kit and pull out the valve.
5) Check if the groove in the valve drive is positioned horizontally and in the lower half of the axis.

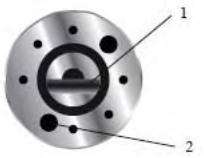


Figure 7.1 Valve plate driving section.
1 = groove
2 = hole for adjustment pins

6) Make sure that the bracket of the new valve is positioned horizontally.

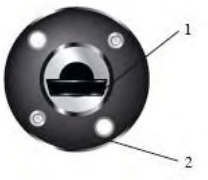


Figure 7.2 Rear view of the autoMACS Pro Separator valve.
1 = bracket
2 = adjustment pins

7) Carefully insert the new valve allowing the bracket to find the groove in the drive. At first, the bracket will slide in only halfway.

Figure 7.3 Inserting the new valve.

8) Gently rotate the valve. The two adjustment pins will slide into their corresponding holes in the valve plate (see Figure 7.1)

9) Make sure that the valve is fully inserted into the driving station. Fasten valve screws using the screwdriver.

10) Connect the tubing with the installed new valve and fasten tubing by hand until finger tight. For details on the connection of tubing, see section 7.8.

11) Plug in and switch ON the autoMACS Pro Separator.

12) Perform a **Rinse** program and check the valves visually for leakage and air inlet.

13) Refasten tubing connectors using caution. If leakage persists call technical service.

14) Take care not to pinch the tubings at the bottom left of the instrument when closing the bottom cover.

> **Note:** In case the valve exchange program **Valve_ex** cannot be performed (e.g. valve does not turn any more) switch off device and turn the valve bracket manually to the position corresponding to the groove in the valve drive.

7.6 Exchange of dilutor valve

1) Switch OFF the instrument

2) Loosen the plunger lock screw.

3) Move the plunger holder downwards.

4) Unscrew the syringe.

5) Unscrew the tubing.

6) Unscrew the two hexagonal socket screws using the key that was delivered with the valve. Take care to note the orientation of the valve shaft as shown below.

7) Pull the valve out of the coupling.

> **Note:** The profile of the valve shaft is shaped asymmetrically, similar to a trapezoid (see Figure 7.4 below).

8) When inserting the new valve into the coupling, make sure that the short side of the trapezoid-shaped valve shaft points towards the triangle in the coupling.

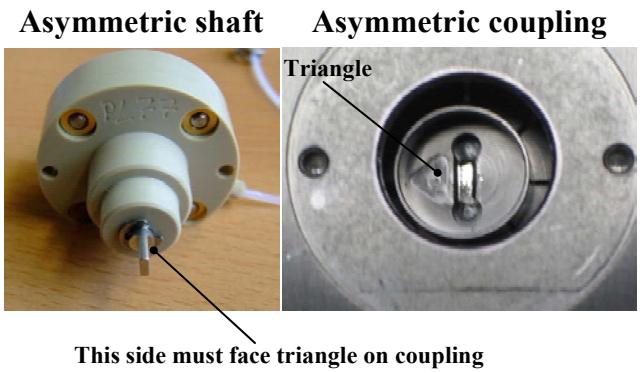


Figure 7.4 Inserting a new valve: take care to note the orientation of the valve.

9) When the valve is properly inserted, mount the hexagonal socket screws.

10) Connect the tubing according to the positioning of the standard valve.

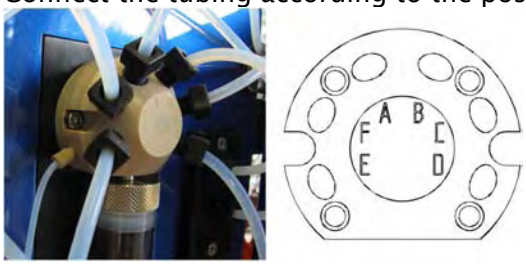


Figure 7.5 Connect tubing to the value as shown above.

11) Guide the draining tube towards the washing station as shown below.

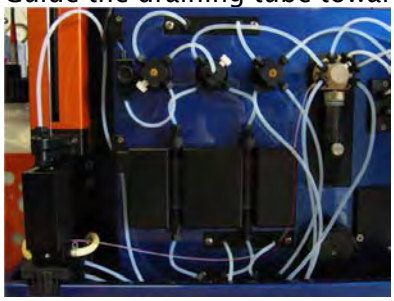


Figure 7.6 Red dashed line indicates the positioning of the draining tube. The draining tube connects the valve to the washing station.

12) Remove the Washing Station. Take care to clean spilled fluids with ethanol or disinfectant.

13) Carefully remove the waste distributor from its position by pulling in an upward direction and remove the blind screw.

Figure 7.7 The waste distributor.

14) Mount the draining tube and finger tighten. Take care to clean spilled fluids with ethanol or disinfectant.

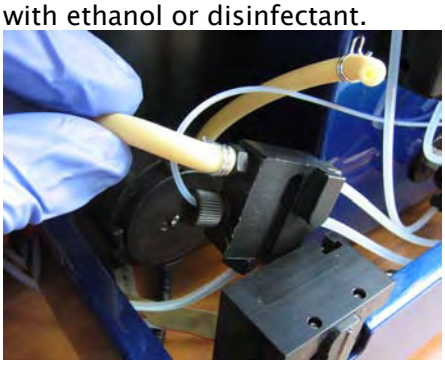


Figure 7.8 The draining tube is attached.

15) Install the waste distributor back to its former position and reinstall the washing station.

16) Install the syringe.

17) Switch on the autoMACS Pro Separator.

18) Check for correct function by running the program **Rinse.**

19) Run the program **Calibr_2** to calibrate the fluidic volume control of the instrument. For details, see section 3.2.7.

7.7 Exchange of autoMACS™ Columns

The autoMACS Columns should be exchanged after 100 separations or 2 weeks after the last column exchange, whichever comes first. The procedure is described in detail in section 3.2.8.

7.8 Exchange of the tubing system

If there is any leakage in the tubing system, the affected tubing part should be exchanged. Please note that each tubing has a specific length and should be exchanged exclusively with the corresponding spare part.

1) Switch off and unplug the instrument.
2) Remove the affected tubing by loosening the tube connectors using the black wrenches.
3) Replace the tubing with the correct part.
4) Pull back the connector from the tubing, so that the tubing can be easily inserted into the appropriate port.
5) Insert the connector cautiously and precisely and fasten it by hand. Make sure not to overwind the screw.
6) Plug in and switch on the instrument. Run the program **Rinse** and check for leakages.
7) Run the program **Calibr_2** to calibrate the volume control of the instrument. For details, see section 3.2.7.
8) If there are any leakages, go to section 10.1.2 for advice on how to improve the tubing connection.

7.9 Exchange of fuses

If the instrument fails to start upon switching it on or if operation suddenly stops and the screen is dark, an exchange of the fuses might be required.

CAUTION: Replace fuses only with those of the same type and rating.

1) Switch the instrument OFF (section 6.7).
2) Unplug the main power cord from the power outlet as well as from the instrument. The fuse holder is located below the main power connector on the rear panel of the instrument.
3) Pull out the fuse holder from the housing and exchange fuses. Fuse specifications are given in section 11.1. and on the marking plate on the rear of the device close to the fuse holder. Do not use other fuses than specified. Push the fuse holder back into the housing and reconnect the main power cord.

7.10 Exchange of hydrophobic air filter

Hydrophobic air filters (0.2 µM) are attached to the bottle closures to vent the liquid bottles and to prevent release of aerosols. To avoid clogging of the filters and to prevent contamination of liquids, air filters should be exchanged if they come into direct contact with any liquid i.e. become wet. They also should be exchanged once a year to avoid clogging through dust deposits.

7.11 Cleaning of washing station

The washing station is designed for the automated rinsing of the outlet and uptake ports as well as surface cleaning of the uptake port needle. The washing station should be cleaned as necessary to remove spills and salt crusts.

1) Switch off and unplug the instrument.
2) Make sure that the needle arm is in the uppermost position.
3) Swivel the front cover to the right side.
4) Swivel the cover of the washing station to the left side. Cover can be removed by lifting it.
5) Press the tubing clamp on the right hand side of the washing station to remove the tubing.

> **Note:** Potentially contaminated liquid may spill out of the orifice of the washing station and the tubing. Therefore, wear protective gloves, protective clothing, and safety glasses to avoid contact with skin and eyes.

6) Unscrew the thumb screw that attaches the washing station to the instrument.
7) Pull out the washing station.
8) Clean the washing station by soaking it in 10% bleach and 70% ethanol for 15 min each. Optionally, sonicate it in water. Rinse with distilled water.

> **Note:** Do not autoclave the washing station or wash using a dish washer.

9) Reassemble the unit in reverse order.

7.12 Exchange of pump head for peristaltic waste pump

The head of the peristaltic waste pump should be exchanged at least once per year.

1) Switch off and unplug the instrument.

2) Remove the washing station as described in 7.11, steps 1–5.

3) Press the tubing clamp on the left hand side of the waste distributor and remove the tubing.

4) Pull out the bottom cover.

5) Press clamps on both sides of the pump and pull out the pump head.

6) This will uncover a pin that protrudes from the instrument. Clean the pin using 70% ethanol but do not attempt to pull it out. The pin drives the pump during operation.

7) Replace pump head with the spare part.

8) Reassemble in reverse order. Take care not to pinch the tubing at the bottom left when pushing the bottom cover back into place.

7.13 Cleaning of uptake/outlet ports

1) Switch off and unplug the instrument.

2) The ports can be wiped with tissue soaked with 70% ethanol, isopropyl alcohol, or MACS Bleach followed by distilled or deionized water.

3) Remove the finger-guard that is attached underneath the outlet port holder by pulling gently. Clean the finger-guard as described for the ports.

4) Wipe the outlet port for the negative fraction as indicated above. The port can be flushed by using a syringe.

5) To clean the uptake port, switch **OFF** the instrument. Move the needle holder up and down to get access to the entire surface of the needle.

6) Push the finger-guard back into position.

> **Note:** To prevent the formation of salt deposits, wipe the outlet ports with a tissue soaked with distilled (or deionized) water before each **Sleep** program.

7.14 Safe program

Depending on the level of use and general instrument maintenance, it is recommended to decontaminate the fluidic system every 3 to 6 months using the **Safe** program. The procedure includes a column exchange.

1) To start the **Safe** program, select menu **Option** from the upper navigation bar on the touchscreen. Then select **Special** and the program **Safe**. Press **Run**.

Subsequently, you will be guided through the decontamination procedure. Follow the prompts displayed on the screen.

2) Disconnect the tubings from all buffer bottles. Press **OK.**

3) Place the ends of the tubings into tubes containing a minimum of 15 mL disinfectant solution (MACS Bleach). Place a tube containing a minimum of 25 mL of disinfectant solution in rack Chill 50 (position A). Press **OK**.

4) The decontamination procedure is performed automatically. Upon completion of the process, replace fluid containers and reconnect all tubings. Press **OK**. The system is rinsed automatically.

5) Subsequently, exchange the columns (see 3.2.8). When finished, press **OK**. The system is rinsed automatically.

6) Clean uptake needle manually using tissue soaked in 70% ethanol. Subsequently, wipe with distilled or deionized water and press **OK**. The system is now ready for separation.

7.15 Decontamination

Please read the chapter “Important information” (section 1) carefully before starting with decontamination.

1) If the instrument has been used to process biohazardous samples, it is recommended to decontaminate the fluidic system by running the **Safe** program (see section 4.1.6).

2) If sample tubes and fraction collection tubes have been in contact with biohazardous material, they should be autoclaved after use.

3) In case of spillage, it is recommended to use a disinfectant that is appropriate for the potential pathogen, e.g. bleach, isopropyl alcohol or 70% ethanol, to decontaminate surfaces with tissue or swabs.

4) The ports of the automated arm and the surface of the instrument also can be decontaminated upon contact with biohazardous samples.

> **Note:** Dispose tissues and swabs appropriately. It is recommended to wear protective gloves, protective clothing, and safety glasses to prevent contact with skin and eyes. Switch off and unplug the instrument beforehand.

5) Bottles and bottle closures can be washed with detergent, 1% hypochlorite, or 70% ethanol.

7.16 Rescue procedure

Should the separation be interrupted before target cells are eluted, it is possible to perform a cell rescue procedure to recover the sample. If the instrument can be restarted, follow procedure A; if the instrument cannot be restarted, follow procedure B.

7.16.1 Rescue procedure A

1) Restart the instrument by switching it off and on again.
2) Undo the tubing connector at the negative port and place into a 50 mL tube.
3) Take out the uptake port needle from the needle holder and place it into a 50 mL tube.
4) Undo the tubing connector of the waste tube at the waste bottle and place it into a 50 mL tube; place a second 50mL tube beside this one.
5) Run the program **Qrinse**. This will rinse the complete fluidic system with autoMACS Pro Running Buffer eluting the cells into the 50 mL tubes. Depending on which step of the separation program that the interruption occurred the cells will be found in any one of the vials.
6) Combine all fractions and centrifuge at 350 x g for 10 min.
7) Discard the supernatant and apply cells to a re-separation as soon as possible. Keep cells on ice until the separation.
8) Re-connect all tubing at the appropriate positions and re-position up-take needle in needle holder

> **Note:** Depending on the nature of your sample it is recommended to wear protective gloves, protective clothing, and safety glasses to prevent contact with skin and eyes. Dispose tissues, swabs and vials appropriately.

7.16.2 Rescue procedure B

If it is not possible to restart the instrument the cells retained on the columns can be recovered.

1) Switch off the instrument and disconnect from the power supply.
2) Prepare two 50 mL tubes in rack and fill two 5 mL syringes with Running Buffer/Separation Buffer.
3) Open the front cover and place absorbent tissue underneath the columns.
4) Pull out the column from the column holder and replace the top connector with a 5 mL syringe filled with Running Buffer.
5) Undo the bottom connector and flush the column into a 50 mL tube. Discard column and syringe appropriately.
6) Repeat steps 4 to 5 with the second column.

7) Centrifuge tubes at 350 x g for 10 min.

8) Discard the supernatant and perform re-separation on the recovered cells as soon as possible. Keep cells on ice until the separation.

9) Install new columns or Dummy columns in place of the discarded ones.

Note: Depending on the nature of your sample it is recommended to wear protective gloves, protective clothing, and safety glasses to prevent contact with skin and eyes. Dispose tissues and swabs and vials appropriately.

7.17 Spare parts list

Part number	Spare part	Part number	Spare part
130-022-101	Pump Seal	130-093-306	autoMACS Pro Bottle Closure, black
130-090-339	Pump Syringe	130-093-307	autoMACS Pro Bottle Closure, red
130-090-378	Square Key	130-093-308	Dilutor Valve, 6-port distribution
130-090-385	Hydrophobic Air Filter	130-093-309	autoMACS Pro Tube (t32)
130-090-386	O-Ring for Bottle Closure	130-093-310	autoMACS Pro Tube (t26)
130-090-387	Air Filter Connector	130-093-311	autoMACS Pro Tube (t20)
130-090-389	Power Cord (D)	130-093-312	autoMACS Pro Tube (t12, t14)
130-090-391	Power Cord (USA)	130-093-313	autoMACS Pro Tube (t3)
130-090-644	Plunger Lock Screw	130-093-314	autoMACS Pro Tube (t18)
130-090-676	Column Connector	130-093-315	autoMACS Pro Tube (t11, t25)
130-090-684	4-port 4-way Valve	130-093-316	autoMACS Pro Tube (t6, t8, t23)
130-090-685	4-port Distribution Valve	130-093-317	autoMACS Pro Tube (t10, 16, 17, 19, 22)
130-090-834	Fuse Holder	130-093-349	Washing Station, needle arm
130-090-835	Column Substitute, set	130-093-350	autoMACS Pro Tube (t1, t13)
130-091-339	Air Filter Extension Set	130-093-362	Tube Connector 2x2 Ports (1/4"-28 UNF)
130-091-996	Valve Blind Screw, 5 pcs.	130-093-364	Tube Guiding Ball, needle arm
130-092-951	MACS Cooling Tube Rack, Chill 5	130-093-365	Peristaltic Pump Head incl. Tube
130-092-952	MACS Cooling Tube Rack, Chill 15	130-093-366	Outlet Port Unit, needle arm
130-092-953	MACS Cooling Tube Rack, Chill 50	130-093-367	Guard, needle arm
130-092-990	MACS Cooling Tube Rack, set	130-093-368	Thumb Screw (M5x16)
130-093-245	autoMACS Pro Angle Connector, set	130-093-370	Magnet Lock, front cover
130-093-246	autoMACS Pro Laminar Hood Plate	130-093-371	4-port 3-way Valve
130-093-284	autoMACS Pro Tube (t27)	130-093-372	autoMACS Pro Tube (t31)
130-093-285	autoMACS Pro Tube (t2)	130-093-397	Uptake Port Needle Guiding, needle arm

Part number	Spare part	Part number	Spare part
130-093-286	autoMACS Pro Tube (t7)	130-093-407	Thumb Screw (M5x40), MiniSampler
130-093-287	autoMACS Pro Tube (t21)	130-093-412	MACS Cooling Tube Rack, 3 x Chill 15
130-093-288	autoMACS Pro Tube (t5)	130-093-413	MACS Cooling Tube Rack, 3 x Chill 50
130-093-289	autoMACS Pro Tube, reservoir (t9, t24)	130-093-416	MACS Cooling Tube Rack, 3 x Chill 5
130-093-290	autoMACS Pro Uptake Port Needle	130-093-532	autoMACS Pro Protection Cover
130-093-291	autoMACS Pro Bubble Sensor	130-093-669	Cover, MiniSampler
130-093-292	MACS Fluid Container (1.5 L)	130-094-289	autoMACS Pro Laminar hood set
130-093-293	autoMACS Pro Tube (t4)	130-094-573	autoMACS Pro Autolabeling Upgrade Kit
130-093-302	Fuse 5x20 T4A, set	130-094-574	MACS Reagent Rack 4
130-093-303	autoMACS Pro Sensor Cable	130-094-682	Pump Syringe, Hamilton, 5ml, Cavro XP
130-093-304	autoMACS Pro Bottle Closure, green	130-094-729	Dilutor Valve, 6-port distribution, V2
130-093-305	autoMACS Pro Bottle Closure, blue		

Table 7.1 List of available spare parts.

8 Protocols

The following chapter describes several cell sorting protocols that may be used with the autoMACS Pro Separator.

8.1 Magnetic labeling instructions

As described above, there are two basic approaches to magnetic labeling: labeling with direct MACS MicroBeads or indirect labeling with MicroBeads against primary antibodies or their conjugates. General instructions for magnetic labeling are described in the following sections.

Note: The exact conditions for magnetic labeling may vary from one type of MACS MicroBeads to another. Detailed protocols and product documentation are provided on the data sheet for the particular MACS Cell Separation Reagents.

Note: Work fast, keep cells cool, and use pre-chilled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

Note: Volumes for magnetic labeling procedures described below are for up to 10^7 total cells. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 total cells, use twice the volume of all indicated reagents and double the total volume). When working with fewer than 10^7 cells, do NOT scale down the volumes.

8.2 Direct magnetic labeling

A variety of antibody-conjugated MicroBeads targeting many human, non-human primate, mouse, and rat cell surface markers are available for direct magnetic labeling. The general protocol given below is based on a 1:5 dilution of MACS MicroBeads. Please note that direct magnetic labeling of mouse cells is typically performed in a 1:10 dilution of MACS MicroBeads. For details, refer to the specific MACS Cell Separation Reagent data sheet.

1) Prepare a single-cell suspension and determine the cell number.
2) Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3) Resuspend cell pellet in 80 µL of fresh buffer per 10^7 total cells.
4) Add 20 µL of MACS MicroBeads per 10^7 total cells.

5) Mix well and refrigerate for 15 minutes at 4–8 °C.

> **Note:** Keeping the samples on ice may result in poor cell labeling. Temperatures higher than 8 °C and/or longer incubation times may lead to non-specific cell labeling.

6) (Optional) Add staining antibodies, e.g. 10 µL of MACS Fluorochrome-conjugated Antibodies, and incubate according to the data sheet.

> **Note:** Fluorescent labeling can be performed either before or after the separation has been completed.

7) Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

8) Resuspend up to 10^8 cells in 500 µL of buffer.

> **Note:** For higher cell numbers, scale up buffer volume accordingly.

9) Proceed to magnetic separation (see section 5.3.5).

8.3 Indirect magnetic labeling

Indirect magnetic labeling is the method of choice, if direct MACS Cell Separation products are not available. Almost any monoclonal or polyclonal primary antibody can be used: A variety of Anti-Fluorochrome MicroBeads is available for magnetic labeling of cells stained with primary antibodies conjugated to fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), allophycocyanin (APC), or tandem molecules like PE-Cy5 or APC-Cy7. Anti-Biotin MicroBeads and Streptavidin MicroBeads are recommended when using biotinylated primary antibodies. Streptavidin MicroBeads must be used with biotin-free buffer for labeling. This is not necessary when using Anti-Biotin MicroBeads, since they do not bind free biotin. Anti-Immunoglobulin MicroBeads against human, mouse, rat, or rabbit immunoglobulins can be used in combination with unconjugated antibodies.

> **Note:** Primary antibodies should be titrated to determine the optimal staining dilution.
>
> **Note:** After incubation with primary antibodies, wash the cells carefully. If unbound primary antibodies have not been completely removed, they may inhibit labeling of cells with indirect MACS MicroBeads.

1) Prepare a single-cell suspension and determine the cell number.

2) Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.

3) Resuspend cell pellet and incubate with the primary antibody according to the manufacturer's recommendations. For MACS Fluorochrome-conjugated or

Biotinylated Antibodies, resuspend up to 10^7 total cells in 100 µL of buffer and add 10 µL of the respective antibodies.

4) Mix well and refrigerate for 5–10 minutes at 4–8 °C or according to the manufacturer's recommendations. If fluorochrome-conjugated antibodies are used, incubate in the dark.

5) Wash cells to remove unbound primary antibody by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

6) (Optional) Repeat washing step.

7) Resuspend cell pellet in 80 µL of buffer per 10^7 total cells and add 20 µL of indirect MicroBeads per 10^7 total cells.

> **Note:** When using Anti-FITC MicroBeads or Streptavidin MicroBeads, resuspend cell pellet in 90 µL of buffer per 10^7 total cells and add 10 µL of MicroBeads per 10^7 total cells.

8) Mix well and refrigerate for 15 minutes at 4–8 °C.

> **Note:** Keeping the samples on ice may result in poor cell labeling. Temperatures higher than 8 °C and/or longer incubation times may lead to non-specific cell labeling.

9) (Optional) When using unconjugated or biotinylated primary antibodies, cells can be stained with a fluorochrome-conjugated antibody at this point.

10) Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

11) Resuspend up to 10^8 cells in 500 µL of buffer.

> **Note:** For higher cell numbers, scale up buffer volume accordingly.

12) Proceed to magnetic separation (see section 5.3.5).

8.4 MACS® MultiSort Kits

MACS MultiSort Kits utilize either direct or indirect magnetic labeling.

8.4.1 Magnetic labeling using direct MACS® MultiSort MicroBeads

A variety of MACS MultiSort Kits is available for sequential positive selection of human cell subsets characterized by multiple cell surface markers.

> **Note:** After release of the MultiSort MicroBeads, a second cell sorting procedure can be performed using direct MACS MicroBeads or indirect MACS MicroBeads, such as Anti-Biotin MicroBeads, Streptavidin MicroBeads, or Anti-Fluorochrome MicroBeads. Anti-Immunoglobulin MicroBeads can also be used, but any reactivity with the isotype of the antibody conjugated with the MACS MultiSort MicroBeads has to be excluded.

1) Prepare a single-cell suspension and determine the cell number.
2) Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3) Resuspend cell pellet in 80 µL of fresh buffer per 10^7 total cells.
4) Add 20 µL of MultiSort MicroBeads per 10^7 total cells, mix well, and refrigerate for 15 minutes at 4–8 °C. For exceptions, see individual MACS MultiSort Kit data sheet.

> **Note:** For labeling with CD34 MultiSort MicroBeads, use the FcR Blocking Reagent (see CD34 MultiSort Kit data sheet).
>
> **Note:** If second parameter sorting is performed indirectly, it is recommended to label the cells for the last 10 minutes simultaneously with the MultiSort MicroBeads for the first parameter sorting and the primary antibody for the second parameter sorting.

5) (Optional) Add fluorochrome-conjugated antibody with the same specificity as the MultiSort MicroBeads at the titer recommended by the manufacturer and refrigerate for further 5 minutes in the dark.
6) Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7) Resuspend up to 10^8 cells in 500 µL of buffer.

> **Note:** For higher cell numbers, scale up buffer volume accordingly.

8) Proceed to magnetic separation (see section 5.3.5). Collect the positive fraction and proceed to removal of the MACS MultiSort MicroBeads.

8.4.2 Magnetic labeling using indirect MACS® MultiSort MicroBeads

If direct MACS MultiSort Kits are not available for the marker of interest, the first cell sorting can be performed indirectly using FITC-, PE-, APC-conjugated or biotinylated primary antibodies and one of the various indirect MACS MultiSort Kits.

> **Note:** After release of the MultiSort MicroBeads, a second cell sorting can be performed using direct MACS MicroBeads or certain indirect MACS MicroBeads. When using Anti-Immunoglobulin MicroBeads for the second sorting step, any reactivity with the isotype of the primary antibody of the

first sorting step and reactivity with the isotype of the antibody conjugated to the MACS MultiSort MicroBeads has to be excluded.

For example: An Anti-FITC MultiSort MicroBead antibody has been used for the first cell sorting step which is of mouse isotype IgG1. Hence, Goat Anti-Mouse IgG MicroBeads or Rat Anti-Mouse IgG1 MicroBeads cannot be used for the second parameter sorting.

Note: Primary antibodies should be titrated to determine the optimal staining dilution. Labeling of the negative population must be avoided.

Note: After incubation with the primary antibodies, wash cells carefully. If unbound primary antibodies have not been completely removed, they may inhibit labeling of cells with indirect MACS MultiSort MicroBeads.

Note: The concentration of indirect MultiSort MicroBeads used to achieve optimal magnetic separation is primarily dependent on the intensity of labeling with the primary antibody and to some degree also on the frequency of target cells in the suspension. Weakly labeled target cells require a higher concentration of indirect MultiSort MicroBeads to achieve optimal magnetic labeling and separation. Target cells with a high frequency (>50%) may also require a higher concentration of indirect MultiSort MicroBeads than target cells with lower frequencies (see table below).

1) Prepare a single-cell suspension and determine the cell number.
2) Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3) Resuspend cell pellet and stain with the primary antibody according to the manufacturer's recommendations. For MACS Fluorochrome-conjugated or Biotinylated Antibodies, typically resuspend 10^7 total cells in 100 µL of buffer and add 10 µL of the antibodies.

   Note: If second parameter sorting is performed indirectly, it is recommended to label the cells simultaneously with the primary antibody for the first parameter sorting and the primary antibody for the second parameter sorting.

4) Mix well and refrigerate for 5–10 minutes at 4–8 °C, or according to the manufacturer's recommendations. If fluorochrome-conjugated antibodies are used, refrigerate in the dark.
5) Wash cells to remove unbound primary antibody by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
6) (Optional) Repeat washing step.
7) Resuspend cell pellet in buffer and add indirect MultiSort MicroBeads according to recommendations in the table below. For more details see also section *Recommendations to optimize cell isolation using indirect MultiSort Kits*.

"weak"	80 µL	20 µL
"intermediate"	90 µL	10 µL
"strong"	96 µL	4 µL

Table 8.1 Buffer and MicroBead volumes required for labeling of cells with varying antigen expression. Volumes are calculated for 10^7 total cells.

> **Note:** Too strong a dilution of the MultiSort MicroBeads may result in poor retention of the labeled target cells. Too weak a dilution may result in less efficient release of MultiSort MicroBeads.

1) Mix well and refrigerate for 15 minutes at 4–8 °C.

> **Note:** Keeping the cells on ice may result in poor cell labeling. Temperatures higher than 8 °C and/or longer incubation times may lead to non-specific cell labeling.

2) Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
3) Resuspend up to 10^8 cells in 500 µL of buffer.

> **Note:** For higher cell numbers, scale up buffer volume accordingly.

4) Proceed to magnetic separation (see section 5.3.5). Collect the positive fraction and proceed with removal of the MACS MultiSort MicroBeads.

8.4.3 Removal of MACS® MultiSort MicroBeads

1) Remove a sample to analyze the separation by flow cytometry and proceed with remaining magnetically labeled fraction.
2) Add 20 µL of MultiSort Release Reagent per 1 mL cell suspension.
3) Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
4) (Optional) To remove any residual magnetically labeled cells, repeat the magnetic separation procedure as described in section 5.3.5.
5) Separate cells using the same autoMACS Pro Program as will be used for the second separation. Collect labeled (non-released) and non-labeled (released) cell fractions to determine the efficiency of the release reaction.

> **Note:** This step is extremely important if the target cells of the second parameter sorting are rare (<10 % target cells in the positive fraction after first separation).

6) Wash cells from the non-labeled (released) fraction carefully by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

7) Resuspend cells in buffer in a final volume of 50 µL per 10^7 total cells.

8) Add 30 µL of MultiSort Stop Reagent per 10^7 total cells and mix well.

9) Add the recommended amount of direct or indirect MACS MicroBeads (see respective MACS Cell Separation Reagent data sheet) to magnetically label the cells for the second marker. Adjust to 100 µL total volume per 10E7 total cells by adding buffer.

10) Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).

11) Continue as described in the respective MACS Cell Separation Reagent data sheet and proceed to section 5.3.5.

8.4.4 Recommendations to optimize cell isolation using indirect MultiSort Kits

The efficiency of the reaction leading to the release of indirect MultiSort Microbeads depends on the intensity of the magnetic labeling. The intensity of the magnetic labeling depends on the efficiency of labeling with the primary antibody for the first sorting step and on the amount of indirect MultiSort MicroBeads used for magnetic labeling (see table above). Typically, a release of greater than 90% is obtained when working with the indirect MultiSort MicroBeads.

$$\text{Release}\,(\%) = 100 \times \frac{\text{No. of cells in released fraction}}{\text{No. of cells in released} + \text{nonreleased fractions}}$$

Excessive magnetic labeling due to high concentrations of indirect MultiSort MicroBeads results in an insufficient release of the MultiSort MicroBeads. An insufficient release decreases the purity of the cells after positive selection with the second parameter.

- Use a lower amount of indirect MultiSort MicroBeads, if the recovery of the target cells after the primary parameter sorting with the indirect MultiSort MicroBeads is sufficient, but the release is lower than 90%.

  Scarce magnetic labeling due to insufficient concentrations of indirect MultiSort MicroBeads results in low recovery of the labeled target cells in the positive fraction.

- Use a higher amount of indirect MultiSort MicroBeads, if the release is efficient (greater than 90%), but the recovery of the positive cells after the first positive selection with the indirect MultiSort MicroBeads is too low.

8.4.5 Direct magnetic labeling of human cells using MACS® Whole Blood MicroBeads

MACS Whole Blood MicroBeads have been developed for positive selection of many human cell types from anticoagulated whole blood samples. Perform magnetic labeling with MACS Whole Blood MicroBeads according to the following general instructions. Refer to the specific MACS Cell Separation Reagent data sheet for more details.

1) Add 50 µL MACS Whole Blood MicroBeads per 1 mL anticoagulated whole blood. When working with larger or smaller blood volumes, scale all reagent volumes and total volumes up or down, accordingly (e.g. for 500 µL of blood use half the volume of Whole Blood MicroBeads, i.e. 25 µL).
2) Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
3) Wash cells by adding 2–5 mL of buffer per ml whole blood and centrifuge at 445×g at room temperature for 10 minutes and slow deceleration (no brake).
4) Aspirate supernatant carefully. Do not disturb the cell pellet. Leave a small residual volume of supernatant (approximately 1–2mm in height) to avoid cell loss.
5) Resuspend the cell pellet, estimate the volume, and dilute with the same volume of buffer and proceed to section 5.3.5.
6) Fluorescent labeling should be performed after the separation process.

9 Quality control of separations performed with the autoMACS™ Pro Separator

To evaluate any MACS Cell Separation, the separated cells can be analyzed with regard to purity, recovery, and viability. Using MACS MicroBeads, the magnetically labeled cells can be simultaneously stained with fluorochrome-conjugated antibodies. Antibodies of the same specificity can be used in most cases. MACS Fluorochrome-conjugated Antibodies are standardized to evaluate MACS Cell Separations. The stained cells can subsequently be analyzed by flow cytometry, fluorescence microscopy, or other techniques.

9.1 Recovery of cells

In most cases, the number of isolated cells will be compared to the number of cells theoretically expected from the heterogeneous starting population. To calculate the target cell recovery, take an aliquot from the magnetically labeled fraction just before starting the cell separation.

The target cell recovery, e.g. positive cells in the magnetically labeled cell fraction can be calculated as follows:

$$\text{Target cell recovery}\,(\%) = 100 \text{ x } \frac{\text{No. of cells in pos. fraction x \% positive cells in pos fraction}}{\text{No. of cells in orig. sample x \% positive cells in orig. sample}}$$

The overall cell recovery can be calculated as follows:

$$\text{Overall cell recovery}\,(\%) = 100 \text{ x } \frac{\text{No. of cells in pos. fraction} + \text{No. of cells in neg. fraction}}{\text{No. of cells in orig. sample}}$$

Note: To count the cells of the original fraction, collect an aliquot of the cell sample after magnetic labeling directly before the magnetic separation to analyze whether cell losses are due to centrifugation steps OR to magnetic separation. Also take counting statistics into consideration. The standard deviation when counting cells is $N \pm N^{1/2}$. Therefore, cell counting might be associated with large statistical errors.

9.2 Purity of isolated cell population

For most experiments that follow the cell separation, it is necessary to document the purity of the isolated cell subset. It is recommended to analyze the cells by flow cytometry. Alternatively, fluorescence microscopy can be used. Cells can also be analyzed by immunocytochemistry.

Purity of the positively selected cell fraction:

$$\text{Purity} = \%\ \text{positive cells in positive (magnetically labeled) fraction}$$

Purity of the depleted cell fraction:

$$\text{Purity} = \%\ \text{negative cells in negative (non-labeled) fraction}$$

Note: Efficient staining of the cells with regard to signal intensity and specificity are the prerequisites for an accurate analysis. Magnetic cell separation using the MACS Technology is a highly sensitive method and often results in extremely pure cell populations. Make sure that the method used for analysis is sensitive enough to accurately analyze the purity.

9.3 Viability of the cells

Different dyes are available to discriminate between live and dead cells. The most common method to discriminate between live and dead cells is based on trypan blue staining and analysis by light microscopy. Trypan blue crosses the cell membrane of dead cells and stains the cells. Live cells are not stained.

Propidium iodide (5.0 µg/mL) is most often used for flow cytometry and fluorescence microscopy. It crosses the permeable cell membrane of dead cells, enters the nucleus, and interacts with DNA. Therefore, the nucleus of dead cells is fluorescently stained. Other fluorescent dyes, for example, DAPI [4',6-diamidino-2-phenylindole] can be used depending on the properties of the flow-cytometer, i.e. its excitation wavelength capabilities, particularly in the UV range.

When working with fixed cells, it is recommended to use the Fixation and Dead Cell Discrimination Kit (#130-091-163) for both the cell fixation as well as the discrimination of dead cells.

The viability can be calculated as follows:

$$\text{Viability}\ (\%) = 100 \times \frac{\text{No. of live cells}}{\text{No. of total cells (live \& dead)}}$$

10 Troubleshooting

The following section aims to help solve problems that might occur while using the autoMACS Pro Separator for cell separation. If the outcome of a cell separation procedure is deemed unsatisfactory, this may either be due to incorrect function of the instrument or to inappropriate sample preparation. Both of these factors are discussed in this section. At the end of this section, a list of numerically encoded errors and warning messages are presented along with user actions for troubleshooting.

Note: It is recommended to use the program **check_up** if general hardware errors occur. Select the **Options** menu from the upper navigation bar. Then select **User settings**. Highlight **check_up** and press **Run**.

The program automatically analyzes the functionality of moving hardware components. A report is displayed after the analysis of each single component.

The procedure can be canceled after each step or continued by pressing **OK**. Following hardware components are analyzed: dilutor valve, valves 1–5, peristaltic waste pump, magnet 1–2, needle arm (movement along the z-axis and the y-axis), and MACS MiniSampler. Furthermore, the calibration data is checked.

10.1 Problems not indicated by error screens

This section addresses problems that are not indicated by a warning or error screen, but might occur during the separation or rinsing programs. Identify the problem and refer to the appropriate section.

10.1.1 Column leakage

1) If a freshly installed autoMACS Column shows signs of leakage, check if the column is installed properly. The column should be inserted precisely into the column connector and fastened to the point of resistance. If this is not the case, loosen the column connector, insert the column precisely, and tighten the connector again.
2) Run the **Qrinse** program: select the **Separation** menu from the upper navigation bar and **Wash now** from the lower navigation bar. Select **Qrinse** and press **Run**. Check if the leakage persists. If so, unscrew the column and check if the luer

connectors of the columns are damaged. If this is the case, exchange the leaking column with a new autoMACS Column (see section 3.2.8).

3) Check if the column connector is fastened properly. If not, use second wrench to counter and tighten another quarter-turn.

4) If the problem persists, contact technical service.

10.1.2 Tubing leakage

1) Identify the location of the leaky tubing by running the **Qrinse** program (see 4.1.6).

2) Check whether the tubing is tightened properly. If this is not the case, tighten the tube connector. The connector should be inserted precisely.

3) If the problem persists, loosen the tube connector and pull back the connector from the tubing.

> **Note:** Do not remove the connector from the tubing.

4) Check the ends of the tubing for wear and fissures. If necessary, replace tubing with the appropriate spare part. Insert the tubing into the appropriate port. Then cautiously insert and fasten the tube connector.

5) Run the **Qrinse** program and check if the leakage persists. If so, unscrew the tubing and check if the screw thread is damaged. If this is the case, order and install new tubing. Please note that each tubing has a specific length and should be exchanged with the corresponding spare part only.

6) If the problem persists, contact technical service.

10.1.3 Pump syringe leakage

Verify that the Running buffer has equilibrated to room temperature before performing a washing or separation program. Cold buffer will make the plunger seal constrict more than usual and may lead to leakage. To clean the pump syringe see section 7.14 and retry. If the problem persists follow the guidelines below.

1) Run the **Sleep** program (see 6.7) and switch off the device.

2) Remove the pump syringe as described in section 7.4.2, points 1 through 4. Remove visible salt crusts from the syringe using distilled water.

3) Carefully remove the plunger from the syringe. Take a tissue and distilled water and clean both the plunger and the syringe. Remove all deposits. Use distilled or deionized water to wet the plunger and carefully push the plunger back into the syringe.

4) Install the pump syringe according to section 7.4.2, points 5 through 7. Make sure that the syringe is installed precisely into the syringe housing.

5) Run a **Qrinse** program to ensure that the problem is solved. If the leakage persists, order and install either a new pump syringe (Order no. 130-090-339) or a new pump seal (Order no. 130-022-101). For details on the installation, see sections 7.4.2.

> **Note:** Depending on the level of use, the pump syringe should be cleaned every 1-3 months.
>
> Appropriate maintenance and long-term storage assures that no salt deposits accumulate in the pump syringe. Salt deposits may cause wear of the pump seal and thus may lead to leakage.
>
> The pump syringe should not run dry at any time. This can damage the pump seal and thereby may lead to leakage of the pump syringe.

10.1.4 Washing station overflow

1) Verify that the Washing station is not clogged with salt deposits. Take out the Washing station and clean as indicated in section 7.11.

2) Reassemble Washing station und run a **Qrinse** program. If problem persists follow the steps below.

3) Make sure that the peristaltic waste pump works properly. Run the **check_up** program (see section 4.1.6).

4) If the **check_up** program reports a problem with the waste pump, remove the pump head and clean the pin that drives the pump (for details, see section 7.12). Clean the washing station (see section 7.11. Reassemble the unit and check whether the problem persists.

5) If the problem persists, replace the pump head (see section 7.12).

10.1.5 MACS® MiniSampler does not move properly

1) Check whether the guiding of the MiniSampler is connected properly to the connector at the autoMACS Pro Separator labeled “External CAN”.

2) Check whether the bolt below the rack detection protrudes from the instrument. If this is the case, push it in and turn it clockwise to lock the bayonet mount.

3) Check the cable connection between MiniSampler and autoMACS Pro Separator. Check for cable damages.

4) Check whether the MiniSampler can freely move to both sides and check for any resistance or collision.

5) If the problem persists, contact technical service.

10.1.6 Output volumes are not correct

1) Check for air inlet and leakages in the fluidic system by running the **Qrinse** program.
2) If tubing are leaking, go to section 10.1.2.
3) If the pump syringe is leaking, go to section 10.1.3.
4) If the column is leaking, go to section 10.1.1.
5) If one or more valves are leaking, go to sections 7.5.
6) Check whether all tubing connections including column connections, bottle connections, and connections at the pump syringe are fastened. Loose connections may allow air to enter the system and therefore affect the performance.
7) If the problem persists, run the **Calibr_2** program. Otherwise contact technical service.

10.1.7 Pump syringe is filled with air during operation

1) If there is any air inlet into the pump syringe during operation, the correct proceeding of a separation will be impaired.
2) Check all tubings that are connected to the fluid bottles. Make sure that all tubings are fastened properly. If a screw thread is damaged, order and install new tubing.
3) Check if the hydrophobic air filters connected to the bottles are clogged. Clogging may cause positive or negative pressure in the fluid bottles, which can lead to pressure problems in the fluidic system. If filters are clogged, replace them with new hydrophobic air filters (see section 7.10).
4) Check if the connections and pump syringes are leaky. Go to section 10.1.3.
5) Check if the uptake port needle is connected correctly and no air inlet is possible. If not, unscrew and check screw threads. If they are undamaged, reinsert precisely and fasten. Then use the wrench to turn an extra quarter-turn. Do not to overwind the screw.
6) If the problem persists, contact technical service.

10.1.8 Outlet port is clogged

1) If the outlet ports are clogged, e.g., due to salt deposits, the elution process might be affected.
2) Wipe the outlet port with a tissue soaked with distilled (or deionized) water.
3) Flush the outlet port manually using a syringe filled with 70% ethanol.

> **Note:** To prevent the formation of salt deposits, wipe the outlet ports with a tissue soaked with distilled (or deionized) water before each **Sleep** program.

10.1.9 Contamination of tubing system

1) Run the **Safe** program (see section 4.1.6).
2) If the problem persists, call technical service.

> **Note:** To prevent contamination, run a **Sleep** program before turning off the device. This occurs automatically if the instrument is turned off as described in section 5.3.7. Make sure to run a wash program after switching on the device.

10.1.10 Low cell viability in final fraction

1) Low cell viability can result from problems during both the cell preparation and the autoMACS Pro Cell Separation. Hence, also check section 10.2.
2) Check the 70% ethanol. Make sure that analytical reagent grade ethanol (without additives) is used to prepare the solvent.
3) Check the Running Buffer. Make sure that the appropriate buffer has been used and that no contamination has occurred.
4) Check Washing Solution. Make sure that the appropriate solution has been used and no contamination has occurred.
5) Check the pump syringe for contamination. If contamination is obvious, refer to section 10.1.3 and change columns afterwards. Check for contamination of the tubing system and refer to the appropriate section.
6) Run the **Safe** program to decontaminate the fluidic system.
7) Check the column status. The autoMACS Columns should be exchanged after 100 separations or 2 weeks after the last column exchange, whichever comes first. The procedure is described in detail in section 3.2.8.
8) If the problem persists, contact technical service.

10.1.11 Low purity of isolated cell population

1) Low purities can be caused by problems during both the cell preparation and the autoMACS Pro Separation. Hence, also check section 10.2.
2) Low purities can also result from using an inappropriate separation program or labeling approach. Please also refer to section 6.
3) Check the exchange date of the autoMACS Columns. Using the autoMACS Columns for longer than two weeks or for more than 100 separations within two weeks may affect purity.
4) Check whether the autoMACS Pro Separator has been stored correctly. Inappropriate storage or allowing the autoMACS Pro Separator fluidic system to run dry will affect the columns and therefore the purity.
5) Make sure that the appropriate Running Buffer has been used.
6) Perform the **Qrinse** program. If pumps are filled with air during the process, refer to section 10.1.7.
7) Perform a test run using PBS as a mock sample and check output volumes. For correct volumes refer to Table 6.2 in section 6.2.3. If volumes are not correct, go to section 10.1.6.
8) Run the **Safe** program (see section 4.1.6).
9) If the problem persists, contact technical service.

When utilizing the autolabeling feature

1) Check whether the uptake needle is positioned in the middle of the sample tube during the mixing procedure. If not, inadequate mixing will be performed.
2) Check whether the reagent volume (contents of reagent vial) is sufficient for labeling.
3) Check that the recommended maximal sample processing time of 90 minutes was not exceeded. In the event of an elevated room temperature the cooling capacity of the Chill Racks may be compromised. Higher labeling temperatures could cause unspecific binding which may lead to lower purities when using positive selection kits.

10.1.12 Low recovery of isolated cells

1) Low recoveries and low purities may be due to similar problems. Go to section 10.1.11, points 1–4.
2) Low recoveries can also be caused by partial column blockage. Run the **Safe** program and exchange the autoMACS Columns (see sections 4.1.6 and 3.2.8).

3) Perform a test run using PBS as a mock sample and check output volumes. For correct volumes refer to Table 6.2 in section 6.2.3. If volumes are not correct, go to section 10.1.6

4) If the problem persists, contact technical service.

Especially by using autolabeling

1) Check whether the uptake needle is positioned in the middle of the sample tube during the mixing procedure. If not, inadequate mixing will be performed.

2) Check whether the reagent volume (contents of reagent vial) is sufficient for labeling.

3) Check that the recommended maximal sample processing time of 90 minutes was not exceeded. In the event of an elevated room temperature the cooling capacity of the Chill Racks may be compromised. Higher labeling temperatures could cause unspecific binding which may lead to lower purities when using positive selection kits.

10.1.13 Touchscreen remains dark

1) Switch OFF the device, wait 5 seconds, and switch ON again. If the autoMACS Pro Separator still does not initialize, go to step 2.

2) Check if the power cord is plugged in correctly and if the electric power is switched on.

3) Replace the fuses (see section 7.9). Spare fuses are included in the autoMACS Pro Separator Starting Kit.

4) If the problem persists, contact technical service.

10.1.14 Disruption of power supply during cell separation

In the unexpected event of a power supply failure during the cell separation procedure, follow the rescue procedure to recover the cell sample trapped in the autoMACS Pro Separator fluidic system.

1) Switch on the autoMACS Pro Separator.

> **Note:** If initial wash (**Rinse** program) is enabled, press **Stop** as soon as the **Status** menu appears on the screen. A window opens displaying that the process is paused. Press **Cancel**.

2) Disconnect the outlet tubing from the waste cap and put the end of the tubing in a 50 mL tube. The rescued cell sample will be rinsed out at the waste port.

3) Select the **Separation** menu and **Wash now**. Select **Qrinse** and press **Run**. The cells will be rinsed out with Running Buffer.

4) Reconnect waste closure and waste bottle.

5) Perform the **Rinse** program to prime the autoMACS Pro Separator.

6) The rescued cell sample can now be centrifuged and subsequently be reprocessed with the autoMACS Pro Separator.

10.1.15 Sample not or only partly taken up

1) If the sample has been taken up only partly, check if the sample contains clumps larger than 1 mm. Continue with the separation of this sample. As soon as the separation is finished, continue with step 2. Before processing any remaining samples, use a Pre-Separation Filter (Order no. 130-041-407) to remove the clumps from the sample.

2) Remove the sample tube. Wipe the uptake port needle with a tissue soaked with 70% ethanol. Run the **Qrinse** program.

3) Check for cell clumps in the tubing system. If cell clumps are suspected, run the **Safe** program.

4) For separating cells that tend to aggregate (e.g. tissue cells), it may be helpful to dilute the sample 1:2.

5) Remove the sample tube and run the **Qrinse** program. Check tubing system and pump syringe for air inlet.

6) Make sure that all tubing are connected properly, especially the tubing connected to the columns and to the buffer bottles.

7) Verify that the uptake port needle is connected correctly and that no air inlet is possible. Tighten if necessary.

8) Make sure that both columns are installed correctly.

9) Clean pump syringe as described in section 10.1.3 to remove salt deposits. Salt crusts might allow air to enter the fluidic system.

10) Check whether the hydrophobic air filters connected to the bottles are clogged. If filters are clogged, replace them with new hydrophobic air filters.

11) Run the **Check_up** program to identify hardware malfunctions.

12) If problem persists, call technical service.

10.1.16 Reagent vial runs dry

As the liquid levels in the reagent vials is not controlled by the system before uptake of reagent, it will not be detected if the reagent volume provided in the vial is sufficient for the current separation process or not. Ensure enough reagent in is present in all

vials for all programmed processes. As the residual volume in the vial is about 20 µl, please be sure to overfill the vial with reagent accordingly.

10.2 Magnetic labeling and separation

10.2.1 Positive selection

Positive cells have not been retained on the column

1) Magnetic labeling of the cells is insufficient because the MicroBead concentration was too low. This may be due to the fact that either too much buffer was added to the cells or too much buffer was left on the cell pellet after centrifugation before adding the MicroBeads. Furthermore, the total number of cells may have been miscounted. Use a ratio of MicroBeads and cells as stated in the MACS Cell Separation Reagent data sheet.
2) Labeling of cells was ineffective due to too much debris and or dead cells in the sample. Debris and dead cells will non-specifically bind to all other components present. Antibodies and MicroBeads will be captured non-specifically and not be able to label the cells in the positive fraction sufficiently any more.
3) Labeling of cells was ineffective due to an incubation temperature lower than recommended. It is recommended to incubate cells in the refrigerator (2–8 °C) for labeling.
4) The number of magnetically labeled cells exceeds the column capacity. Calculate the number of magnetically labeled cells, for example, by staining with flurochrome-conjugated antibodies and subsequent fluorescence analysis. The number should not exceed 2×10^8 cells per sample. If necessary, split the sample.
5) Cells were not labeled with MicroBeads because the MicroBeads were degraded. Check the expiration date. Check for sterility of the MicroBeads, if the vial has been opened before.
6) Cells were labeled, e.g., with fluorochrome-conjugated antibodies, prior to magnetic labeling with direct MicroBeads. When antibodies recognizing the same epitope are used for fluorescent and magnetic labeling, fluorochrome-conjugated antibodies and MicroBeads might compete for the binding sites. This can result in insufficient magnetic labeling. It is recommended to perform magnetic labeling prior to staining with fluorochrome-conjugated antibodies. Alternatively, use indirect MicroBeads.

Especially by using autolabeling

1) Check whether the uptake needle is positioned in the middle of the sample tube during the mixing procedure. If not, inadequate mixing will be performed.
2) Check whether the reagent volume (contents of reagent vial) is sufficient for labeling.

Low purity of magnetically labeled cell fraction

1) Check for cell aggregates. Negative cells may be retained when forming clusters with positive cells and, thus, contaminate the positive fraction. Use buffers devoid of Ca^{2+} and Mg^{2+} in order to reduce formation of cell aggregates.
2) Dead cells in the cell suspension may non-specifically bind to MicroBeads and will then be co-enriched in the positive fraction. Remove dead cells before separation by using the MACS Dead Cell Removal Kit (# 130-090-101) or by Ficoll Paque™ density gradient.
3) The concentration of MicroBeads or the temperature used for magnetic labeling were too high. It is recommended to dilute the MicroBeads according to the data sheet. Cells should be incubated with MicroBeads in the refrigerator (2-8 °C).
4) The total number of cells may have been miscounted leading to an inappropriate ratio of MicroBeads and cells. Use a ratio of MicroBeads and cells as stated in the MACS Cell Separation Reagent data sheet.
5) Incubation time with MicroBeads was too long, leading to background labeling. Reduce the incubation time to the recommended values (see individual MACS Cell Separation Reagent data sheets). Typically, an incubation time of 15 minutes is required when incubating in the refrigerator (2-8 °C). For exceptions, see individual data sheets.
6) When target cells are extremely rare (<5% of total cells), few non-labeled cells which are retained non-specifically may constitute a high portion compared to the target cells. Perform a second separation to remove the non-specifically retained cells.

Especially by using autolabelling

1) Check that the recommended maximal sample processing time of 90 minutes was not exceeded. In the event of an elevated room temperature the cooling capacity of the Chill Racks may be compromised. Higher labeling temperatures could cause unspecific binding which may lead to lower purities when using positive selection kits.
2) Check if enough reagent is available for the programmed cell separation.

Recovery of magnetically labeled cells is low
See section “Positive cells have not been retained on the column”.

Viability of magnetically labeled cells is low
Dead cells have been co-enriched in the positive fraction due to non-specific binding. Remove dead cells before separation by using the MACS Dead Cell Removal Kit. See also section 10.1.10.

Overall recovery is low
Cells are often lost during washing steps. Determine the number of cells immediately before magnetic separation to check whether the low recovery is due to this or due to problems during the cell separation.

10.2.2 Depletion

Too many cells are retained on the column

1) Magnetic labeling of the cells was non-specific. In order to block non-specific binding, use MACS FcR Blocking Reagent (# 130-059-901) before labeling.
2) The concentration of MicroBeads and/or the temperature used for magnetic labeling were too high. It is recommended to dilute the MicroBeads according to the data sheet. Cells should be incubated with MicroBeads in the refrigerator (2–8 °C).
3) Incubation time with MicroBeads was too long, leading to background labeling. Reduce the time for incubation to the recommended values (see individual MicroBeads data sheets). Typically, an incubation time of 15 minutes is required at 2–8 °C. For exceptions, see individual MACS Cell Separation Reagent data sheets.
4) Dead cells in the cell suspension may bind non-specifically to MicroBeads and will then be co-enriched in the positive fraction. Remove dead cells before separation by using the MACS Dead Cell Removal Kit (# 130-090-101) or by Ficoll Paque™ density gradient.

Especially by using autolabelling

1) Check that the recommended maximal sample processing time of 90 minutes was not exceeded. In the event of an elevated room temperature the cooling capacity of the Chill Racks may be compromised. Higher labeling temperatures could cause unspecific binding which may lead to lower purities when using positive selection kits.

Non-labeled fraction shows low purity

1) Magnetic labeling of the cells is insufficient. Refer to point 1 in section 9.2.1.1.
2) Magnetic labeling was insufficient due to a low expression level of the surface marker. It is recommended to use an indirect magnetic labeling system (for example MACS Anti-Biotin MicroBeads) in order to increase magnetic labeling of the cells.
3) The number of magnetically labeled cells exceeds the column capacity. Calculate the number of expected magnetically labeled cells. The number of cells should not exceed 2×10^8 per sample. If necessary, split the sample.
4) Labeling of cells was ineffective due to an incubation temperature lower than recommended. Incubate the cells in the refrigerator (2–8 °C) for labeling.
5) Labeling of cells was ineffective due to too much debris and or dead cells in the sample. Debris and dead cells will non-specifically bind to all other components present. Antibodies and MicroBeads will be captured non-specifically and not be able to label the cells in the positive fraction sufficiently any more.
6) Cells were not labeled with MicroBeads because the MicroBeads were degraded. Check the expiration date. Check for sterility of the MicroBeads, if the vial has been opened before.

Especially by using autolabeling

1) Check whether the reagent vial has enough reagent to perform magnetic cell labeling.
2) Check whether the uptake needle is positioned in the middle of the sample tube during the mixing procedure. If not, limited mixing could lead to low purity.
3) Refer to the respective data sheet for the recommended separation program.

Recovery of target cells is low

Refer to section 10.2.1.

Overall recovery is low

Refer to section 10.2.1.

10.2.3 Indirect MACS® MicroBeads

Note: Indirect magnetic labeling of the cells strongly depends on the quality of the primary antibody and the efficiency of labeling with the primary antibody.

Positive cells have not been retained on the column

1) The unbound primary antibody was not completely removed and inhibits magnetic labeling with indirect MACS MicroBeads. Wash cells carefully (optionally, wash twice) by adding 1–2 mL of buffer per 10^7 cells after incubation with primary antibody. Centrifuge and remove supernatant completely.
2) The concentration of the primary antibody is too low. Therefore, magnetic labeling is not sufficient to retain the desired cells on the column. To avoid this, the primary antibody should be titrated carefully. For tips and hints on titration, see the FAQs under Customer Support on our website at www.miltenyibiotec.com.
3) The isotype of the primary antibody is not recognized by the Anti-Immunoglobulin MicroBeads. Make sure to use appropriate MicroBeads.
4) Check the expression of the antigen/epitope with flow cytometry.
5) Antibodies may have degraded. Check the antibody for its function. Storage of diluted reagents at 4 °C or –20 °C may lead to degradation
6) Cells were not labeled with MicroBeads because the MicroBeads were degraded. Check the expiration date. Check for sterility of the MicroBeads, if the vial has been opened before.Antibodies may have degraded. Check the antibody for its function. Storage of diluted reagents at 4 °C or –20 °C may lead to degradation.

Positive fraction shows poor purity

1) The concentration of the primary antibody was too high, leading to non-specific binding of unwanted cells. The primary antibody should be titrated carefully. For tips and hints on titration, see the FAQs under Customer Support on our website at www.miltenyibiotec.com.
2) The primary antibody shows non-specific binding. Add blocking reagents such as BSA or immunoglobulin before labeling the cells.
3) The incubation time during labeling was too long. Do not exceed the incubation times specified in the particular data sheet.
4) Check the primary antibody for its specificity. When antiserum is used, it is recommended to preadsorb the antiserum, e.g., on cells which do not express the antigen, or to purify it by affinity chromatography, ammonium sulfate precipitation, ion chromatography, etc. in order to avoid non-specific cross-reaction.

10.3 Fluorescent staining

Note: Generally, MACS Fluorochrome-conjugated Antibodies are used in a 1:11 dilution (resuspend up to 10^7 cells in 100 µL of buffer and add 10 µL of MACS Fluorochrome-conjugated Antibodies). For details, refer to the MACS Reagent data sheet. If other than MACS Antibodies are used, carefully titer staining reagents. For tips and hints on titration, see the FAQs under Customer Support on our website at www.miltenyibiotec.com.

10.3.1 Cells are poorly stained

1) Fluorochrome-conjugated antibodies may have deteriorated. Store MACS Fluorochrome-conjugated Antibodies at 2–8 °C.
2) Fluorochrome-conjugated antibodies and MACS MicroBeads might compete for epitopes. Check the efficiency of the fluorescent staining on cell samples that are not magnetically labeled. Especially for weakly expressed antigens, it might be necessary to use a fluorochrome-conjugated antibody that is directed against an epitope different from that recognized by the MicroBeads.
3) For indirect staining of biotinylated antibodies with streptavidin-fluorochromes, please note that buffers supplemented with fetal bovine serum or bovine serum albumin may contain free biotin which can inhibit streptavidin binding to the biotinylated antibody. Alternatively, use MACS Anti-Biotin-FITC, -PE, or -APC. MACS Anti-Biotin antibodies do not bind to free biotin.

10.3.2 Cells are excessively stained:

1) High background staining may occur when the concentration of the staining reagents is too high and the incubation time for the staining procedure is too long. The use of the pre-titrated MACS Fluorochrome-conjugated Antibodies is recommended to achieve optimal results.
2) Check the staining reagent for its specificity.

10.4 Problems indicated by error or warning screens

If errors or warnings are displayed on the screen of the device, please refer to the following table. If the below measures do not clear the fault, call Miltenyi Biotec Technical Service. To assist in the troubleshooting process please have the instrument serial number and details of the error message at hand (i.e. error number, module number, file and error line). If this is not possible, please go to the instrument **Log List** and view the **Log Details** taking care to note the display parameters exactly.

Error Code	Cause	Possible remedies
-5	Hardware module is not initialized. After a module malfunction an initialization of the module is neccessary. This might also be a subsequent error if another error has been	Restart instrument. Please call technical service if error is thrown again.

Error Code	Cause	Possible remedies
	displayed shortly before.	
-27	Standard valve initialization failed. Valve may be worn or blocked and cannot be turned correctly or valve drive is damaged.	Touch button DETAILS. Exchange displayed valve. If error is displayed again call technical service.
-28	Motor rotation detection failed. Valve may be worn or blocked and cannot be turned correctly. Otherwise valve or magnet drive malfunction.	Touch button DETAILS. Exchange displayed valve if applicable. If error is displayed again or magnet is displayed call technical service.
-29	Valve rotation hindered. Valve may be worn or blocked and cannot be turned correctly.	Touch button DETAILS. Exchange displayed valve if applicable. Make sure to preparate samples as specified. If error is displayed again call technical service.
-257	Needle arm movement has been hindered.	If object blocked movement, remove object. Switch off device. Wait 5 seconds. Switch on device. Otherwise or if error is displayed again call technical service.
-263	Unable to read rack bar code correctly	Check bar code on rack or try different rack. Make sure ambient or direct sun light does not hit sensor.
-264	Unable to read rack bar code correctly	Check bar code on rack or try different rack. Make sure ambient or direct sun light does not hit sensor.
-769	Diluter plunger could not be initialized. Syringe not mounted correctly, syringe damaged or diluter valve not positioned correctly.	Check if syringe is fastened tightly. Exchange if broken or damaged. If error is displayed again call technical service.
-775	Diluter is not initialized. After a diluter malfunction an initialization of the module is necessary. This might also be a subsequent error if another error has been displayed shortly before.	Restart instrument. Please call technical service if error is thrown again.
-777	Plunger movement blocked because of column clogging, blocked tubing set or any other cross-section constriction	Restart instrument and try a Qrinse to wash out clogged material. If error persists run Safe program. Otherwise exchange diluter valve or standard valves depending on where a constriction is suspected. Please call technical service if error is displayed again.
-6006	Air intake during sample uptake although needle did not yet hit bottom of tube. Leakage of air into system in front of bubble sensor, liquid level has been overestimated or needle did not move to bottom (as fast as neccessary).	Make sure foam on top of sample is not higher than 5 mm above liquid level. Check for leakage at transition of needle to tubing and tubing to bubble sensor.
-6009	Unexpected air in system during sample uptake.	Check for leakage or air bubbles in tubing from uptake-needle to valve 1. Calibrate needle arm. Check for buffer supply. Otherwise call technical service.
-6216	The reagent designated for the current labeling process is not assigned a position in the reagent rack.	Provide all necessary reagents in reagent rack und correct reagent rack definition.
-7001	Needle could not be retreated completely.	Try Reinitialization by touching RETRY. Otherwise call technical service.
-7002	Collision of the needle with the bottom of the tube (or any other object) has been detected, but resetting the collision sensor failed although the needle has been lifted.	Check for smooth running of the needle in its support then touch CONTINUE. Otherwise calibrate needle arm, especially if using a chill 15 rack.
-7003	Collision of the needle with an unexpected object. Resetting the collision sensor failed as the needle could not be lifted (already too close to top).	Check for objects hindering the movement of the needle. Remove the uptake needle from the needle holder and verify that there are no physical obstructions. Press CONTINUE.
-7004	Collision of the needle with an unexpected object far above expected tube bottom.	Check that cover of the washing station is properly closed. Remove any objects hindering the movement of the needle. If needle hits rim of tube or top of rack calibrate needle arm using Calibr_1 program. Touch LIFT for needle retreat.
-7005	Collision of the needle with an unexpected object far above expected tube bottom.	Check that cover of the washing station is properly closed. Remove any objects hindering the movement of the needle. If needle hits rim of tube or top of rack calibrate needle arm. Touch RETRY to try again.
-7006	Collision of the needle with an unexpected object far above expected tube bottom.	Check that cover of the washing station is properly closed. Remove any objects hindering the movement of the

Error Code	Cause	Possible remedies
		needle. If needle hits rim of tube or top of rack calibrate needle arm. Touch RETRY to try again.
-7007	Sample volume exceeds maximum volume specified for rack or program type. Remaining portion of sample will not be processed.	Do not use sample volumes exceeding the maximum volume specified for rack or program type. If volumes are in specified range but error is displayed anyway, please call technical service.
-7008	The sample volume has been underestimated. The needle has been rinsed but might still be contaminated.	Please clean outside of needle manually. If this error is thrown frequently please call technical service.
-7009	Collision of needle with bottom of tube could not be detected at expected position.	Check if tubes are correctly positioned in rack corresponding to template programming. Check MACS MiniSampler connection in front of autoMACS Pro Separator.
-7010	Not certain if liquid surface of sample has been detected correctly.	Touch IGNORE to continue without liquid detection. Needle will be moved to bottom directly. This might result in a subsequent warning -7008 if the liquid level is higher than 60 mm above the tube bottom (see above). TOUCH retry to continue with liquid detection. Ensure that the tubing from the needle arm to bubble sensor can move freely. Adjust the tubing if necessary. Otherwise call technical service.
-7011	Restart of the device is required.	Please restart device.
-7012	Calibration data not found.	Please calibrate needle arm axes. Run program Calibr_1.
-7013	Calibration data not found.	Please calibrate needle arm axes.
-7014	Calibration data not found.	Please calibrate tubing. Run program Calibr_2.
-7015	Columns are not installed.	Please install columns.
-7018	Calibration data not found.	Please calibrate MACS MiniSampler using program Calibr_1.
-7021	A separation program has been started but system had not been rinsed properly.	Please rinse system by touching WASH or abort with CANCEL.
-7022	Columns are overdue.	Please install new columns. Touch CANCEL to abort and then install columns, touch CONTINUE to ignore and use old columns (not recommended).
-7023	Plunger movement blocked because of column clogging, blocked tubing set or any other cross-section constriction during output of the negative fraction. The negative fraction has not been eluted completely. Negative cells are still remaining in the system.	Touch CONT to discard the remaining negative cells into the waste bottle. To output negative fraction again at lower speed, exchange negative tube with an empty tube and touch RETRY.
-7024	The number of programmed sample positions exceeds the actual positions of the rack on the MACS MiniSampler.	Exchange rack with rack holding more samples and touch OK or touch CANCEL to abort and reprogram.
-7026	The protective cover of the MACS MiniSampler seems to be opened by the moving needle arm hitting the cover.	Check configuration and connection of the protective cover, the MACS MiniSampler and the front support at the autoMACS Pro Separator then touch CONTINUE. Touch CANCEL to abort.
-7027	The protective cover of the MACS MiniSampler needs to be closed.	Please close protective cover and touch CONTINUE. Touch CANCEL to abort.
-7028	Bar code on chill rack could not be read. MACS MiniSampler is not connected (properly).	Check electrical connection of MACS MiniSampler. If detected the MACS MiniSampler symbol would be displayed in the status screen.
-7029	Unable to read rack bar code correctly	Check bar code on rack or try different rack. Make sure ambient or direct sun light does not hit sensor. Touch RETRY to try again, touch SELECT to set chill rack type without automatic bare code reading.
-7030	The device has been shut down without using SLEEP.	Always use SLEEP to shut down the device.
-7031	The given whole blood sample size exceeds the maximum volume specified. The sample cannot be diluted sufficiently.	Do not use sample volumes exceeding the maximum whole blood sample volume specified for the used chill rack type. If volumes are in specified range but error is displayed anyway, please call technical service.
-7032	Air in system during calibration of tubing.	Check buffer supply. Check for leakage of system (unintended air intake). Start a rinse program (WASh ONLY) and then retry calibration. Otherwise call technical service.

Error Code	Cause	Possible remedies
-7033	Date and time is outdated.	Set time and date to actual values.
-7034	Air intake during sample uptake although needle did not yet hit bottom of tube. Leakage of air into system in front of bubble sensor, liquid level has been overestimated or needle did not move to bottom (as fast as neccessary).	Make sure foam ontop of sample is not higher than 5 mm above liquid level. Check for leakage at transition of needle to tubing and tubing to bubble sensor. Touch CONT to process currently uptaken sample volume. Touch CANCEL to abort.
-7035	Air intake during sample uptake although needle did not yet hit bottom of tube. Leakage of air into system in front of bubble sensor, liquid level has been overestimated or needle did not move to bottom (as fast as neccessary). Uptaken sample has been processed. Portion of sample is remaining in tube.	Make sure foam ontop of sample is not higher than 5 mm above liquid level. Check for leakage at transition of needle to tubing and tubing to bubble sensor.
-7036	Plunger movement blocked because of column clogging, blocked tubing set or any other cross-section constriction during output of the positive fraction. The positive fraction has not been eluted completely. Positive cells are still remaining in the system.	Touch CONT to discard the remaining positive cells into the waste bottle. To output positive fraction again at lower speed, exchange positive tube with an empty tube and touch RETRY.
-7037	Resuspensed sample could not be taken up completely (no final air intake detected). Leakage of air into system behind bubble sensor. Portion of resuspended sample is remaining in tube.	Check for air inlet into system behind bubble sensor. Touch CONT to process currently uptaken sample volume. Touch CANCEL to abort.
-7038	Resuspensed sample could not be taken up completely (no final air intake detected). Leakage of air into system behind bubble sensor. Portion of resuspended cells are remaining in tube.	Check for leakage of air into system behind bubble sensor.
-7039	Required volume cannot be provided in given chill rack.	Exchange rack with rack able to provide requested volume (e. g. chill 50 instead of chill 15). Touch RETRY to try again. Touch CANCEL to abort.
-7048	Miscalculation of diluter movement. Target position is negative.	Touch RETRY to use target position 0 instead of negative value for current diluter move. Touch CANCEL to abort. Please contact the technical service in all cases - also if the sample has been processed completely after touching RETRY.

11 Technical data and specifications

The technical specifications of the autoMACS Pro Separator and the peripheral devices are as follows:

11.1 Technical data and specifications of the autoMACS™ Pro Separator

Model	autoMACS Pro Separator
Input voltage	100–240 VAC, ~50/60 HZ
Power consumption	200 VA
Fuses	2×T4A/250
RS232-Interface (labeled “COM”)	Pins 1: 4, 6, 9 NC Pin 2: RXD Pin 3: TXD Pin 5: GND Pin 7: RTS Pin 8: CTS
RS232-Interface (labeled “RS232/AUX”) Not in use	Pins 1, 4, 6, 7, 8, 9: NC Pin 2: RXD Pin 3: TXD Pin 5: GND
RS232-Interface + DC-Output (labeled “RS232/BCR”)	Pins 4, 6: NC Pin 1: Input Pin 2: RXD Pin 3: TXD Pin 5: GND Pins 7, 8: Shorted Pin 9: 5 VDC / 0.5 A
CAN-Bus + DC-Output (labeled “External CAN”)	Pins 1, 4, 8: NC Pin 2: CAN-L Pins 3, 6: GND Pins 5, 9: 24 VDC / 2A Pin 7: CAN-H
AC-Output (labeled “Bottle Sensor”)	Pins 1, 2, 3, 4, 5: 5 VAC / 10 kΩ Pins 6, 7, 8, 14, 15: GND Pins 9, 10, 11, 12, 13: Input
CAN-Bus (labeled “CAN1” or “CAN2”)	Pins 1, 4, 5, 8, 9: NC Pin 2: CAN-L Pins 3, 6: GND Pin 7: CAN-H

Table 11.1 Technical data and pin assignment for autoMACS Pro Separator

The autoMACS Pro Separator is labeled as a protection class I device and must be plugged into a grounded power outlet, see Important information (section 1).
The main power supply cord and plug of the instrument shall comply with following specifications (USA and Canada only): UL listed and KAM cord, minimum type SJ,

minimum 18 AWG, 3 conductors. Rated for a minimum temperature of 60 °C. Provided with grounding-type (NEMA 5-15P) attachment plug, rated 125 Vac, 10 A. Opposite end terminates in IEC 320 style connector, rated 125 Vac, 10 A.

Parameter	Specification
Color	Blue / orange
Footprint	605 mm × 343 mm (w × d)
Footprint with MiniSampler	605 mm × 455 mm (w × d)
Height	392.5-454 mm (adjustable touchscreen)
Weight	25 kg
Input voltage (autoMACS Separator)	100-240 VAC, 50-60 Hz
Input voltage (autoMACS MiniSampler)	24 Vdc
Power consumption	200 VA
Programs	12 preset
Sample volume (input)	0.25-50 mL
Sample volume (output)	0.5-52 mL
Column capacity	4×10^9 cells / sample 2×10^8 magnetically labeled cells / sample 15 mL of whole blood

Table 11.2 Technical data and pin assignment for the autoMACS Pro Separator

Conditions of operation: 15-30 °C with 0-85% humidity at a maximum altitude of 2000 m. Supply voltage fluctuations up to +/-10% of the nominal voltage. Transient overvoltages present on the mains supply: category II. The instrument is suitable for rated pollution degree 2.

The autoMACS Pro Separator is not specified for use in the cold room. The autoMACS Pro Separator has been investigated by Underwriters Laboratories in accordance with the standards UL 61010-1, CAN/CSA- C22.2 No. 61010-1, and IEC 61010-2-081 and meets the intent of the directive 2004/108/EC (electromagnetic compatibility) and 2006/95/EC (low voltage equipment).

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Compliance was demonstrated by conformance to the following standards which have been listed in the Official Journal of the European Communities:

EMC:	EN 61326-1
	EN 61000-3-2
	EN 61000-3-3
Low voltage equipment:	EN 60825-1
	EN 61010-1
	EN 61010-2-081

Compliance was demonstrated by conformance to the following FCC Rules of the Code of Federal Regulations:
47 CFR §15, class B.

11.2 Technical data and specifications of the MACS® MiniSampler

Parameter	Specification
Model	MACS MiniSampler
Input voltage	24 VDC
Current	0.8 A
Sub D9 interface with shielding	Pin 1: NC Pin 2: CAN- Pin 3: GND Pin 4: NC Pin 5: +24 V Pin 6: GND Pin 7: CAN+ Pin 8: NC Pin 9: +24 V

Table 11.3 Technical data and pin assignment for MACS MiniSampler

The MACS MiniSampler is labeled as a protection class III device and must only be plugged into the connector labelled with “External CAN” of the autoMACS Pro Separator, see chapter warnings and precautions.
Size without lid: 182 mm × 148 mm × 47 mm
Size with lid: 280 mm × 153 mm × 172 mm
Weight: 1.5 kg

The MACS MiniSampler is designed for operation with three different tube racks and a reagent rack.

Rack type	Slots	Maximum number of samples	Maximum sample volume	Maximum number of cells per tube
Chill 5	24 × 5 mL	6 (5 mL tubes)	2.5 mL	5×10^8
Chill 15	15 × 15 mL 5 × 5 mL	5 (15 mL tubes)	12.5 mL	2.5×10^8
Chill 50	6 × 50 mL 3 × 15 mL 3 × 5 mL	3 (50 mL tubes)	50 mL	4×10^8

Table 11.4 MACS Cooling Tube Racks: Chill Racks 5, 15 and 50

Rack type	Slots
MACS Reagent Rack 4	4 × MACS Reagent vials

Table 11.5 MACS Reagent Rack 4

Conditions of operation: 15–30 °C with 0–85% humidity at a maximum altitude of 2000m.
The MACS MiniSampler is not specified for use in the cold room. The MACS MiniSampler has been investigated by Underwriters Laboratories in accordance with the standards UL 61010-1 and CAN/CSA -C22.2 No. 61010-1 and meets the intent of the directive 2004/108/EC (electromagnetic compatibility).

CE c UL US

Compliance was demonstrated by conformance to the following standards which have been listed in the Official Journal of the European Communities:

EN 61326-1

EN 61000-3-2

EN 61000-3-3

Compliance was demonstrated by conformance to the following FCC rules of the Code of Federal Regulations:
47 CFR §15, class B.

12 Technical service

Miltenyi Biotec offers a full range of customer technical support options for your autoMACS Pro Separator.

For support and technical questions, or if you think your autoMACS Pro Separator is defective, please contact your local Miltenyi Biotec representative or Miltenyi Biotec's technical support team:

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13 Limited warranty

Except as stated in a specific warranty statement, which may accompany your autoMACS Pro Separator (the “Product”), or unless otherwise agreed in writing by an authorized representative of Miltenyi Biotec, Miltenyi Biotec’s warranty, if any, with respect to this Product is subject to the terms and conditions of sale (the “Terms”) of the company within the Miltenyi Biotec group which supplied the Product. The Terms may vary by country and region. Copies of these Terms are available on request or at www.miltenyibiotec.com.

Nothing in this document should be construed as constituting an additional warranty.

Miltenyi Biotec‘s product warranty only covers Product issues caused by defects in material or workmanship encountered during ordinary use, as described in the user manual or other documentation provided by Miltenyi Biotec; it does not cover Product issues not arising out of defects in material or workmanship, including but not limited to Product issues resulting from: failure to follow installation, operating and/or maintenance instructions, or environmental conditions prescribed in, this user manual or other Product documentation; misuse; abuse; neglect; mishandling; unauthorized or improperly performed maintenance or repairs; accident; acts of God; limitations of technology; electrical current fluctuations; modification of or to any part of the Product; use of accessories, spare parts and/or consumables other than those recommended by Miltenyi Biotec; or normal wear and tear. Miltenyi Biotec‘s product warranty does not cover products sold AS IS or WITH ALL FAULTS, or which had its serial number defaced, altered or removed, or any consumables, or parts identified as being supplied by a third party; those third-party accessories or parts may be covered by a separate warranty from their manufacturer.

Miltenyi Biotec must be informed immediately, if a claim is made under such warranty. If a material or manufacturing defect occurs within the warranty period, Miltenyi Biotec will take the appropriate steps to restore the full usability of your Product.

Limitation on damages:
Miltenyi Biotec shall not be liable for any incidental or consequential damages for breach of any express or implied warranty or condition on this Product.

Some states or jurisdictions do not allow the exclusion or limitation of incidental or consequential damages, so the above limitations or exclusions may not apply to you. This warranty statement gives you specific legal rights and you may have other rights, which may vary from county to country or jurisdiction to jurisdiction.

14 Glossary

Air filter: Hydrophobic 0.2 µm air filter attached to the bottle closure. Used to vent the bottle and—at the same time—prevent contaminants from entering the fluid bottle.

Air filter connector: Luer-to-thread connector for attaching the air filter to the threaded bottle closure vent.

APC: Allophycocyanin

autoMACS Columns: Specifically designed autoMACS Columns; reusable for 14 days or for 100 separations within these 14 days.

autoMACS Column 1: First autoMACS Column in which labeled cells are retained during positive selection and depletion programs. The autoMACS Column 1 occupies the left slot of the black magnet cover.

autoMACS Column 2: The second autoMACS Column is used during double selection programs. The autoMACS Column 2 occupies the right slot of the black magnet cover.

autoMACS Pro Separator: Automated magnetic cell separator, also referred to as device or instrument.

autoMACS Running Buffer: Sterile and ready-to-use solution for cell separation and washing programs. The tubing connector is color-coded blue.

autoMACS Pro Washing Solution: Sterile and ready-to-use solution for washing and special rinsing programs. The tubing connector is color-coded green.

Bottle closure: Vented screw-on closure with fluid uptake ports / canules and distribution tubes. The bottle closures contain fluid sensors and are equipped with sensor cable connectors.

Column Connector: Luer-to-thread connector connecting the autoMACS Columns to the fluidic system.

Column substitute: Column without spheres that replace the autoMACS Columns for long-term storage and shipment. Column substitutes cannot be used for cell separations.

Depletion: Isolation of target cells by labeling all cells other than the target cells (non-target cells?) with MACS MicroBeads and subsequently performing a MACS Separation. The non-labeled fraction contains the target cells.

Deplete: Depletion program, standard mode: a normal cell deposition rate is used. The non-labeled cells are eluted in row B of the sample rack. This is the optimal program to be used in combination with MACS Cell Isolation Kits or to achieve the highest recovery rate of untouched cells.

Depletes: Depletion program, sensitive mode: a slow cell deposition rate is used (1 mL/min). The non-labeled cells are eluted in row B of the sample rack. This program is optimized for depletion of those cells that weakly express the antigens used for magnetic labeling, or to achieve optimal purity of the untouched cell fraction.

Depl05: Special depletion program: the cell deposition rate is 0.5 mL/min. The non-labeled cells are eluted in row B of the sample rack. This program is used for strong depletion of those cells that weakly express the antigens used for magnetic labeling, or to achieve the highest purity of the untouched cell fraction. However, choosing Depl05 might result in a reduced recovery of the target cell fraction.

Depl025: Special depletion program: the cell deposition rate is 0.25 mL/min. The non-labeled cells are eluted in row B of the sample rack. This program is used for very strong depletion of those cells that weakly express the antigens used for magnetic labeling, or to achieve the highest purity of the untouched cell fraction. However, choosing Depl025 might result in a reduced recovery of the target cell fraction.

FITC: Fluorescein isothiocyanate

Fluid container: 1.5 L bottles holding the fluids for operational use of the autoMACS Pro Separator. Fluid sensors monitor the fluid levels in the containers for Running Buffer, Washing Solution, and waste via electrolyte conductivity. The fluid level in the container for the 70% ethanol cannot be monitored as no electrolytes are present in the solvent.

Fluid sensors: This sensor type measures electrolyte conductivity and is an integral part of the bottle closures of the fluid containers for waste, Running Buffer and Washing Solution.

Fluid sensor cable: Cable connecting the fluid sensor to the autoMACS Pro Separator. The sensor cable connectors on the bottle closures are color-coded: red for waste, green for Washing Solution, and blue for Running Buffer.

Fraction collection tube: 5 mL, 15 mL, or 50 mL plastic sample tubes to collect the positive and negative fractions. The process has been optimized using tubes from BD Falcon.

Front door: The front door opens sidewards, giving access to the autoMACS Columns, pumps, valves, washing station, and tubings.

MACS Technology: Technology developed by Miltenyi Biotec for immunomagnetic labeling and subsequent separation of cells or biomolecules in a high-gradient magnetic field.

MACS MicroBeads: Super-paramagnetic particles conjugated to antibodies for magnetic labeling of cells or biomolecules.

Magnet cover: Black cover surrounding the magnets. The magnet cover is located in the center of the fluidic system and has slots for the autoMACS Columns.

Memory card: Removable compact flash/SD RAM card containing the autoMACS Pro Programs.

Memory card slot: Slot located on the right hand side of the autoMACS Pro Touchscreen, giving access to the memory card. The memory card should be removed by trained personnel only.

Negative fraction: Sample fraction containing the non-labeled cells that pass through the autoMACS Column while the column is placed in the magnetic field.

PE: Phycoerythrin

Ports: The automated arm carries two ports: the port located proximal to the instrument is designed for computer-controlled fluid detection and distribution such as magnetic labeling, sample mixing, sample uptake and release of the magnetically labeled fraction while the port in the front releases the non-labeled fraction.

Positive fraction: Sample fraction containing the cells labeled with MACS MicroBeads. These cells are retained on the column while the column is placed in the magnetic field. The cells are eluted from the column after the column has been removed from the magnet.

Positive selection: Process of isolating cells by labeling the target cells with MACS MicroBeads and performing a MACS Separation. The labeled target cells are eluted in row C of the tube rack.

Possel: Positive selection program in standard mode using one autoMACS Column. The target cells are eluted in row C of the sample rack. This program is used for cells with normal to high frequency and with normal antigen expression.

Possel_s: Positive selection program, sensitive mode, using one autoMACS Column. The target cells are eluted in row C of the sample rack. This program is used for cells

with normal to high frequency which weakly express the antigens used for magnetic labeling, or to achieve the highest recovery of the target cells.

Posseld: Positive selection program, normal mode, using both autoMACS Columns. The target cells are eluted in row C of the sample rack in a volume of 0.5 mL. This program is used to isolate rare cells or to achieve a higher purity of the target cells.

Posseld2: Special positive selection program, normal mode, using both autoMACS Columns. The target cells are eluted in row C of the sample rack in a volume of 2 mL. This program is used to isolate rare cells from whole blood, cord blood, or large cell samples, and to achieve a higher recovery of the target cells.

Posselds: Positive selection program, sensitive mode, using both autoMACS Columns. The target cells are eluted in row C of the sample rack in a volume of 2 mL. This program is used to isolate rare cells that weakly express antigens used for magnetic labeling (e.g. CD133).

Posselwb: Special positive selection program using both autoMACS Columns for the isolation of cell subsets from of whole blood. The target cells are eluted in row C of the sample rack.

Running Buffer bottle: Container for Running Buffer. The bottle closure is equipped with a fluid sensor. The closure, the fluid sensor cable, and the tubing connector are color-coded blue.

Safe solution: Solution of 1% (w/v) sodium hypochlorite in distilled water used to decontaminate the autoMACS Pro fluidic system with the **Safe** program. The safe solution is fed into the system from a 50 mL tube. Upon completion of the **Safe** program, the fluidic system contains Running Buffer.

Storage Solution: Solvent used during the Sleep, Store, Safe, and Column Exchange programs to minimize the contamination risk. The fluidic system of the autoMACS Pro Device is filled with MACS Storage Solution (70% ethanol).

Storage Solution bottle: Container for 70% ethanol. The bottle closure, the fluid sensor cable, and the tubing connector are color-coded black.

Store program: Prior to long term storage, the **Store** program should be applied. During this procedure the autoMACS Columns are replaced with substitutes. Upon completion of the **Store** program, the fluidic system contains 70% ethanol.

Syringe pump: Computer-controlled high precision syringe pump with Teflon® seal plunger that drives fluids through the autoMACS Pro fluidic system.

Touchscreen: High resolution TFT color touchscreen located on top of the autoMACS Pro Separator. The touchscreen is used to operate and monitor the instrument through on-screen menus.

Tubing connector: Plastic threaded connector with square nut used to connect the tubings to the bottle closures, the columns, the pump, or valves.

Tubing system: Permanent set of Teflon® tubing through which fluids circulate in the autoMACS Pro Separator fluidic system.

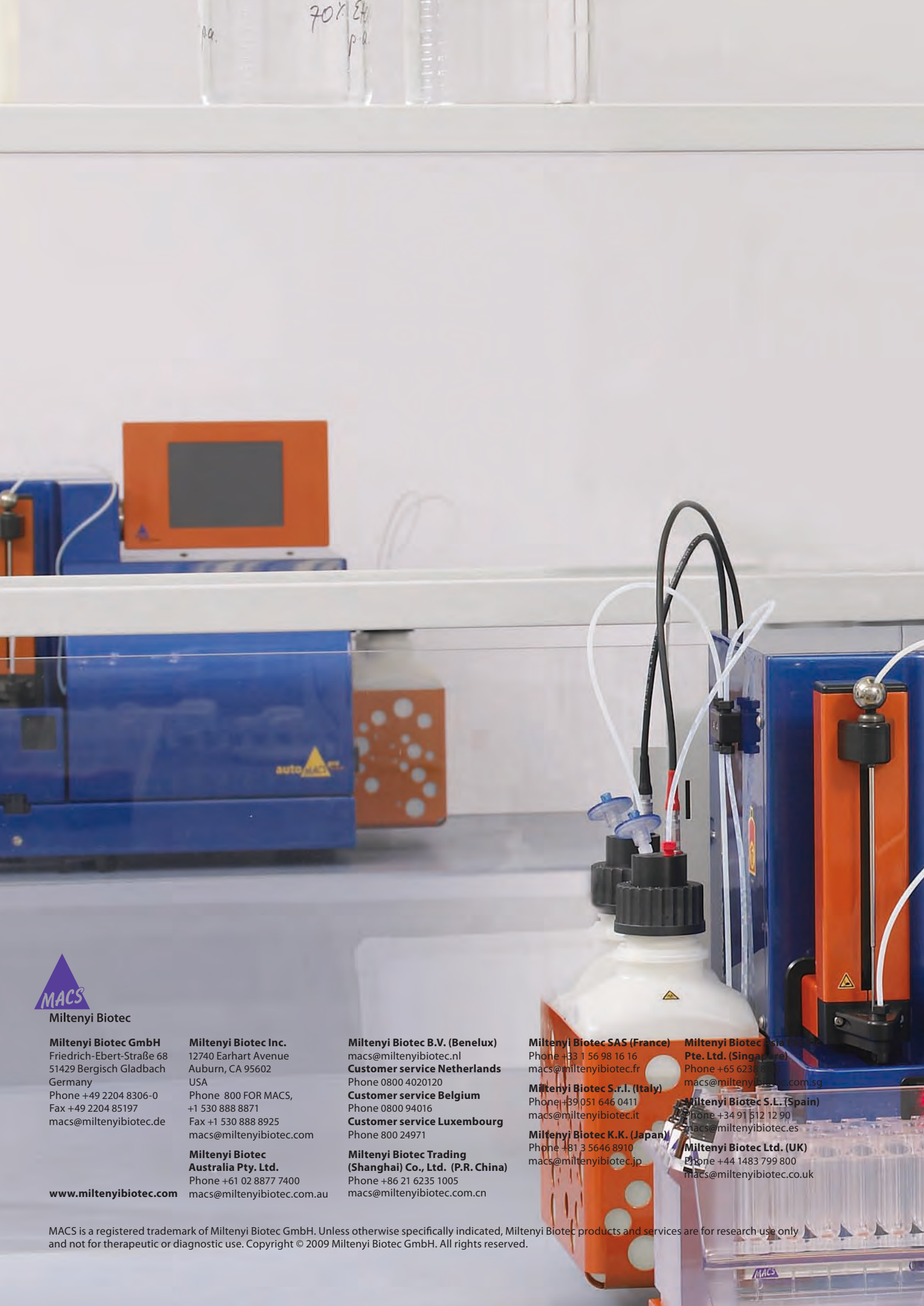
Tube racks: Three different acrylic tube racks are provided with the instrument. They are designed for optimal positioning of sample tubes and fraction collection tubes at the ports of the automated arm. They contain a coolant allowing to pre-cool the racks in the refrigerator for cooling of the cells during the separation process. The racks have four tube positions for each sample. Position **A** holds the sample tube containing the starting material. Position **B** holds the tube for the non-labeled fraction. Position **C** holds the tube for the magnetically labeled fraction. Position **D** is auxiliary. For details, see the table in section 4.1.2.

Washing Solution bottle: Container for autoMACS Pro Washing Solution. The bottle closure is equipped with a fluid sensor. The closure, the fluid sensor cable, and the tubing connector are color-coded green.

Waste container: Container for waste fluid. The closure is equipped with a fluid sensor. The closure, the fluid sensor cable, and the tubing connector are color-coded red.

Whole Blood MicroBeads: MACS MicroBeads developed for isolating target cells directly from whole blood by using the autoMACS Pro Separator.

Wrench: Black wrench used to tighten and loosen tubing connections.



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