Miltenyi Biotec

Mouse Cell Depletion Kit

Order no. 130-104-694

Contents

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

1. Description

This product is for research use only.

Components	2 mL Mouse Cell Depletion Cocktail
Capacity	For up to 2×10^8 tumor cells or up to 1×10^9 total cells (including red blood cells), up to 100 separations.
Product format	Mouse Cell Depletion Cocktail is supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of the MACS® Separation

First, the mouse cells are magnetically labeled with a cocktail of monoclonal antibodies conjugated with MACS® MicroBeads. Then, the cell suspension is loaded onto a MACS Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled mouse cells are retained within the column. The unlabeled human cells run through. After removing the column from the magnetic field, the magnetically retained mouse cells can be eluted.

1.2 Background information

The Mouse Cell Depletion Kit has been designed for the enrichment of untouched human cells upon xenotransplantation. Human tumor xenografts represent the benchmark for research areas like drug discovery, cancer stem cell biology, and metastasis prediction[1]. During the growth phase *in vivo*, xenografted tissue is vascularized and infiltrated by cells of mouse origin, including heterogeneous lymphocyte subpopulations, fibroblasts, and endothelial cells. The level of infiltration is highly dependent on multiple factors like tumor subtype, growth rate, and region of transplantation. However, even when these factors are kept constant, the amount and composition of infiltrating mouse cells are highly variable, which makes accurate molecular downstream analyses difficult. The contaminating mouse cells lead to cross-hybridization of mouse-derived molecules to human probes on microarrays and a significant reduction of sensitivity caused by measurement of mouse signals during next-generation sequencing or proteome analysis[2]. In addition, the culture of human tumor cells is frequently hampered by murine fibroblasts overgrowing the target cells. For optimal results, the Mouse Cell Depletion Kit should be used in combination with the Tumor Dissociation Kit, human (# 130-095-929) and gentleMACS™ Dissociators.

1.3 Applications

- Enrichment of human cells after xenotransplantation.
- Culture or direct use of human cells for biochemical, physiological, pharmacological, and morphological studies.

1.4 Reagent and instrument requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, and 0.5% bovine serum albumin (BSA) by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with PBS. Keep buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column. Always use freshly prepared buffer.
  Do not use autoMACS® Running Buffer or MACSQuant® Running Buffer as they contain a small amount of sodium azide that could affect the results.
- MACS Columns and MACS Separators: For optimal purity and recovery the use of an LS Column is strongly recommended. . Depletion can also be performed by using the autoMACS Pro or the MultiMACS™ Cell24 Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
LS	4×10^7	5×10^7	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
autoMACS	5×10^7	10^8	autoMACS Pro
Multi-24	2×10^7	2.5×10^7	MultiMACS Cell24

▲ **Note:** Column adapters are required to insert certain columns into the VarioMACS™ or SuperMACS™ II Separators. For details refer to the respective MACS Separator data sheet.

Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macs@miltenyibiotec.de
www.miltenyibiotec.com

Miltenyi Biotec Inc.
2303 Lindbergh Street, Auburn, CA 95602, USA
Phone 800 FOR MACS, +1 530 888 8871, Fax +1 877 591 1060
macs@miltenyibiotec.com

140-004-507.06

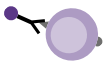
- Tumor Dissociation Kit, human (# 130-095-929) for the generation of single-cell suspension from tumor tissues.
- gentleMACS™ Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Octo Dissociator with Heaters (# 130-096-427)
- gentleMACS C Tubes (# 130-093-237, # 130-096-334).
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD326 (EpCAM)-PE. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells without fixation.
- (Optional) Pre-Separation Filters (30 µm) (# 130-041-407) to remove cell clumps.
- (Optional) Labeling Check Reagent conjugated to, e.g., APC to evaluate purity of sorted cells.

2. Protocol

2.1 Sample preparation

For preparation of a single-cell suspension from xenograft tumors use the Tumor Dissociation Kit, human (# 130-095-929) in combination with the gentleMACS Dissociators.

For details refer to www.gentleMACS.com/protocols.



2.2 Magnetic labeling

▲ Cells can be labeled with MACS® MicroBeads using the autolabeling function of the autoMACS® Pro Separator. For more information refer to section 2.4.

▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ Volumes for magnetic labeling given below are for up to 2×10^6 tumor cells or 10^7 total cells including red blood cells. When working with fewer cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 4×10^6 tumor cells or 2×10^7 total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic labeling. Pass cells through 30 µm nylon mesh (Pre-Separation Filters (30 µm) # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

▲ The recommended incubation temperature is 2–8 °C. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice may require increased incubation times.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 80 µL of buffer per 2×10^6 tumor cells or 10^7 total cells (including red blood cells).
4. Add 20 µL of Mouse Cell Depletion Cocktail per 2×10^6 tumor cells or 10^7 total cells (including red blood cells).
5. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
6. (Optional) Add staining antibodies according to manufacturer's recommendations.
7. Adjust volume to 500 µL using buffer for up to 2×10^6 tumor cells or up to 10^7 total cells.

   ▲ **Note:** Up to 1×10^7 tumor cells or up to 5×10^7 total cells can be processed on one LS Column. If more cells were used split the sample onto multiple LS Columns.
8. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

▲ Always wait until the column reservoir is empty before proceeding to the next step.

Magnetic separation with LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details refer to the respective MACS Column data sheet.
2. Prepare column by rinsing with 3 mL of buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells, representing the enriched human tumor cells.
3. Wash column with 2×1 mL of buffer. Collect unlabeled cells that pass through, representing the enriched human tumor cells, and combine with the flow-through from step 3.

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
5. (Optional) Remove column from the separator and place it on a suitable collection tube.
6. (Optional) Pipette 3 mL of buffer onto the column. Immediately flush out the magnetically labeled mouse cells by firmly pushing the plunger into the column.

Magnetic separation with the MultiMACS™ Cell24 Separator

Refer to the the MultiMACS™ Cell Separator user manual for instructions on how to use the MultiMACS Cell24 Separator.
For a standard separation choose the following program: **Deplete**

2.4 Cell separation with the autoMACS® Pro Separator

▲ Refer to the user manual for instructions on how to use the autoMACS® Pro Separator.

▲ All buffer temperatures should be ≥10 °C.

▲ For appropriate resuspension volumes and cell concentrations, please visit www.automacspro.com/autolabeling.

▲ Place tubes in the following Chill Rack positions:

position A = sample, position B = negative fraction,

position C = positive fraction.

140-004-507.06

2.4.1 Fully automated cell labeling and separation

1. Switch on the instrument for automatic initialization.
2. Go to the **Reagent** menu and select **Read Reagent**. Scan the 2D barcode of each reagent vial with the barcode scanner on the autoMACS® Pro Separator. Place the reagent into the appropriate position on the reagent rack.
3. Place sample and collection tubes into the Chill Rack.
4. Go to the **Separation** menu and select the reagent name for each sample from the **Labeling** submenu (the correct labeling, separation, and wash protocols will be selected automatically).
5. Enter sample volume into the **Volume** submenu. Press **Enter**.
6. Select **Run**.

2.4.2 Magnetic separation using manual labeling

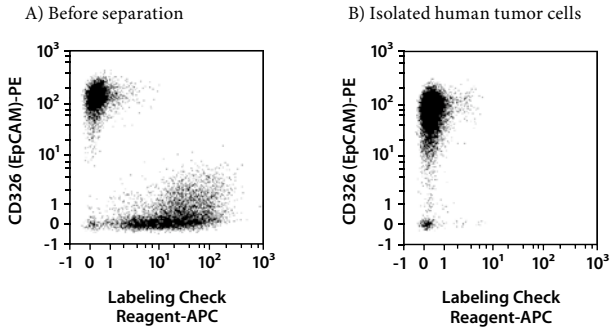
1. Label the sample as described in section 2.2 Magnetic labeling
2. Prepare and prime the instrument.
3. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample and collection tubes into the Chill Rack.
4. For a standard separation choose the following program:

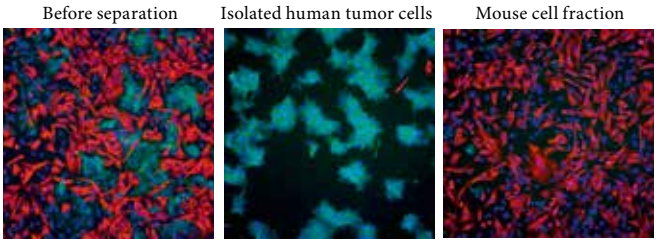
   Depletion: Depletes

   Collect negative fraction in row B of the tube rack. This fraction represents the enriched human tumor cells.

3. Example of a separation using the Mouse Cell Depletion Kit

EpCAM-positive human colon carcinoma cells were isolated from a heterogenous cell population using the Mouse Cell Depletion Kit, an LS Column, and a QuadroMACS™ Separator after the tissue was dissociated using the Tumor Dissociation Kit, human and the gentleMACS™ Octo Dissociator. The figure below shows the sample before separation (A) and the isolated human tumor cells after the mouse cells have been depleted (B). The Labeling Check Reagent-APC was used to analyze the mouse cells.
Subsequently, the unseparated fraction, the negative fraction, and the positive fraction were cultured for three days in expansion medium (90% RPMI 1640, 10% FBS, 100 U/mL penicillin, and 100 U/mL streptomycin). After fixation, cells were stained using antibodies specific for vimentin (red) and human EpCAM (green). Cell nuclei were stained with DAPI (blue).





4. References

1. Rubio-Viqueira, B. and Hidalgo, M. (2009) Direct *in vivo* xenograft tumor model for predicting chemotherapeutic drug response in cancer patients. Clin. Pharmacol. Ther. 85: 217–221.
2. Wong, S. Q. *et al.* (2013) Targeted-capture massively-parallel sequencing enables robust detection of clinically informative mutations from formalin-fixed tumours. Sci. Rep. 3: 3494. DOI: 10.1038/srep03494.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols.