Miltenyi Biotec

Tumor Cell Isolation Kit
mouse

Order no. 130-110-187

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	1 mL Non-Tumor Cell Depletion Cocktail, mouse
Capacity	For up to 5×10^8 total cells (including red blood cells), up to 50 separations.
Product format	Non-Tumor 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 non-tumor 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 non-tumor cells are retained within the column. The unlabeled tumor cells run through. After removing the column from the magnetic field, the magnetically retained non-tumor cells can be eluted.

1.2 Background information

The Tumor Cell Isolation Kit, mouse has been designed for the enrichment of untouched tumor cells from mouse tumors. During the growth phase *in vivo*, tumor tissue is vascularized and infiltrated by cells of non-tumor origin, including heterogeneous lymphocyte subpopulations, fibroblasts, and endothelial cells.
The level of infiltration is highly dependent on multiple factors like tumor subtype, growth rate, site of tumor growth, and status of the host animal. However, even when these factors are kept constant, the amount and composition of infiltrating cells remain widely unpredictable. Molecular downstream analysis as well as culture of mouse tumor cells is frequently challenged by non-tumor cells. Additionally, there is a lack of markers exclusively expressed on mouse tumor cells, which makes direct isolation or identification in analyses like flow cytometry difficult. For optimal results, the Tumor Cell Isolation Kit, mouse should be used in combination with the Tumor Dissociation Kit, mouse (# 130-096-730) and gentleMACS™ Dissociators.

1.3 Applications

- Enrichment of mouse tumor cells from induced mouse tumors.
- Culture or direct use of mouse tumor 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.

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.

- Tumor Dissociation Kit, mouse (# 130-096-730) for the generation of single-cell suspension from tumor tissues.

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- 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) MACS SmartStrainers (70 µm) (# 130-098-462) to remove clumps after dissociation.
- (Optional) Pre-Separation Filters (70 µm) (# 130-095-823) to remove cell clumps.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD44-VioBlue® (# 130-102-443) and Anti-Ter119-PE (# 130-102-336). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Labeling Check Reagent conjugated to, e.g., APC (# 130-095-237) to evaluate purity of sorted cells.

2. Protocol

2.1 Sample preparation

For preparation of a single-cell suspension from mouse tumors use the Tumor Dissociation Kit, mouse (# 130-096-730) in combination with the gentleMACS Dissociators.

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

2.2 Magnetic labeling

▲ 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 10^7 total cells (including red blood cells). When working with fewer than 10^7 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 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 70 µm nylon mesh (Pre-Separation Filters (70 µm), # 130-095-823) 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 10^7 total cells (including red blood cells).

   ▲ **Note:** Always use freshly prepared buffer.
4. Add 20 µL of Non-Tumor Cell Depletion Cocktail per 10^7 total cells.
5. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
6. Adjust volume to 500 µL using buffer for up to 10^7 total cells.

   ▲ **Note:** 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.
7. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells. For details refer to table in section 1.4.

▲ 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 tumor cells.
4. Wash column with 2×1 mL of buffer. Collect unlabeled cells that pass through 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. Pipette 3 mL of buffer onto the column. Immediately flush out the magnetically labeled non-tumor 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

▲ 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.

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.

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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**.

For more details on complete walk away automation on the autoMACS Pro Separator, please refer to the user manual.

2.4.2 Magnetic separation using manual labeling

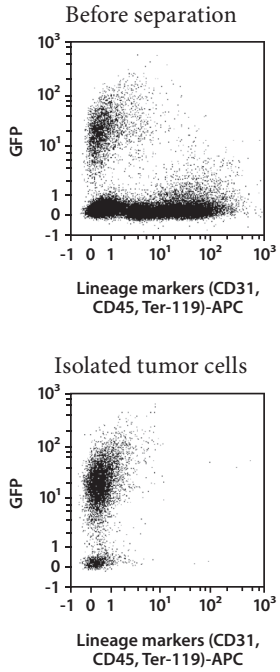
1. Label the sample as described in section 2.2 Magnetic labeling
2. Prepare and prime the instrument.
3. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample and collection tubes into the Chill Rack.
4. For a standard separation choose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

   Depletion: Deplete

   Collect negative fraction in row B of the tube rack. This fraction represents the enriched tumor cells.
5. (Optional) Collect positive fraction from row C of the tube rack. This fraction represents the magnetically labeled non–tumor cells.

3. Example of a separation using the Tumor Cell Isolation Kit

Mouse breast carcinoma cells were purified from a mouse 4T1 tumor using the Tumor Cell Isolation Kit, an LS Column, and a QuadroMACS™ Separator. The 4T1 tumor cells were GFP-labeled prior to tumor induction for subsequent detection. Cells were fluorescently stained with lineage markers (CD45, Ter119, CD31 conjugated to APC) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



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