Miltenyi Biotec

Tumor Cell Isolation Kit
human

Order no. 130-108-339

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 A, human** **1 mL Non-Tumor Cell Depletion Cocktail B, human**
Capacity	For up to 10^8 tumor cells or up to 5×10^8 total cells (including red blood cells), up to 50 separations.
Product format	All components are 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 human 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, human has been designed for the enrichment of untouched human tumor cells from primary specimens. 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, and affected organ site. However, even when these factors are constant, the amount and composition of infiltrating cells are highly variable, which makes accurate molecular downstream analyses difficult. The contaminating non-tumor cells lead to hybridization of non-tumor cell derived mRNA molecules to probes on microarrays and a significant reduction of sensitivity caused by measurement of irrelevant signals during next-generation sequencing or proteome analysis. In addition, the culture of human tumor cells is frequently hampered by fibroblasts overgrowing the target cells. For optimal results, the Tumor Cell Isolation Kit, human should be used in combination with the Tumor Dissociation Kit, human (# 130-095-929) and gentleMACS™ Dissociators.

1.3 Applications

- Enrichment of human tumor cells from primary specimens.
- Culture or direct use of human 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 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
MultiMACS	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.

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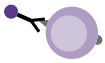
- Tumor Dissociation Kit, human (# 130-095-929) for the generation of single-cell suspension from tumor tissues.
- gentleMACS™ Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Octo Dissociator with Heaters (# 130-096-427)
- gentleMACS C Tubes (# 130-093-237, # 130-096-334).
- (Optional) 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 without fixation.
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD326 (EpCAM)-VioBlue® and CD31-PE. 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 human 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

▲ When working with sarcomas, mesenchymal ovarian carcinoma, or mesenchymal glioblastoma, do not use the Non-Tumor Cell Depletion Cocktail B for the separation. For details please refer to steps 3 and 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 and/or up to 1×10^7 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 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 up to 2×10^6 tumor cells or up to 10^7 total cells in 60 µL of buffer.
   When working with sarcomas, mesenchymal ovarian carcinoma, or mesenchymal glioblastoma, resuspend the cells in 80 µL of buffer.
   ▲ **Note:** Always use freshly prepared buffer.
4. Add 20 µL of the Non-Tumor Cell Depletion Cocktail A and 20 µL of the Non-Tumor Cell Depletion Cocktail B.
   When working with sarcomas, mesenchymal ovarian carcinoma, or mesenchymal glioblastoma, add only 20 µL of the Non-Tumor Cell Depletion Cocktail A. Do not use the Non-Tumor Cell Depletion Cocktail B!
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 2×10^6 tumor cells or up to 10^7 total cells.
   ▲ **Note:** Up to 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.
7. 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.
4. Wash column with the 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**

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2.4 Cell separation with the autoMACS® Pro Separator

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

▲ Buffers used for operating the autoMACS Pro Separator should have a temperature of ≥10 °C.

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

▲ When using the autolabeling function (2.4.1) all samples can either be labeled with the Non-Tumor Cell Depletion Cocktail A and B or only with the Non-Tumor Cell Depletion Cocktail A. Mixing samples, e.g., one sample with Cocktail A and B and one sample with Cocktail A, in the same run is not possible.

2.4.1 Cell separation with the autoMACS® Pro Separator using autolabeling for a fully automated procedure

1. Turn on the instrument for automatic initialization (automated preparation and priming procedure).
2. Program autolabeling by selecting Read Reagent in the "reagent menu" tab and scan the 2D barcode on each reagent vial with the barcode scanner on the autoMACS® Pro instrument. Place the reagent into the appropriate space on the reagent rack.
3. Place sample and collection tubes into the sample rack. Sample tube should be in row A, and the collection tubes in rows B and C.
4. 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.
6. Select run. Wait until the instrument asks if the Non-Tumor Cell Depletion Cocktail B is required or not.

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

2.4.2 Magnetic separation with the autoMACS® Pro Separator 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 tube in row A of the tube rack and the fraction collection tubes in rows B and C.
4. For a standard separation choose the following program:

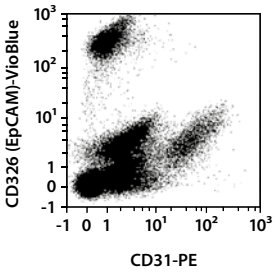
   Depletion: Depletes

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

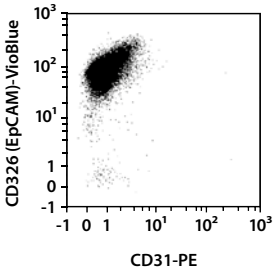
3. Example of a separation using the Tumor Cell Isolation Kit

EpCAM$^+$ human breast carcinoma cells were isolated from a heterogeneous sample of human cells using the Tumor Cell Isolation Kit, human, an LS Column, and a QuadroMACS™ Separator after the tissue was dissociated using the Tumor Dissociation Kit, human and the gentleMACS™ Octo Dissociator. The cells were fluorescently stained with CD326 (EpCAM)-VioBlue and CD31-PE 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.

Before separation



Isolated human tumor cells



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