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## 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.**

<b>Components</b>	1 mL CD45 (TIL) MicroBeads, human: MicroBeads conjugated to monoclonal anti-human CD45 antibodies (isotype: human IgG1).
<b>Capacity</b>	For $5 \times 10^8$ total cells, up to 50 separations.
<b>Product format</b>	CD45 (TIL) MicroBeads are supplied in buffer containing stabilizer and 0.05% sodium azide.
<b>Storage</b>	Store protected from light at $2-8^\circ\text{C}$ . Do not freeze. The expiration date is indicated on the vial label.

### 1.1 Principle of the MACS® Separation

First, the CD45<sup>+</sup> cells are magnetically labeled with CD45 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 CD45<sup>+</sup> cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD45<sup>+</sup> cells. After removing the column from the magnetic field, the magnetically retained CD45<sup>+</sup> cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the CD45<sup>+</sup> cells must be separated over a second column.

### 1.2 Background information

CD45 (TIL) MicroBeads, human have been developed for the isolation of tumor-infiltrating leukocytes (TILs) from single-cell suspensions of solid human tumors as well as xenotransplanted tumors with a humanized immune system. The CD45 antigen is expressed on all cells of hematopoietic origin except erythrocytes and platelets.

### 1.3 Applications

- Positive selection of CD45<sup>+</sup> leukocytes from single-cell suspensions of solid human tumors as well as xenotransplanted tumors with a humanized immune system.

### 1.4 Reagent and instrument requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS® BSA Stock Solution (#130-091-376) 1:20 with autoMACS® Rinsing Solution (#130-091-222). Keep buffer cold ( $2-8^\circ\text{C}$ ). Degas buffer before use, as air bubbles could block the column.

▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS). Buffers or media containing  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  are not recommended for use.

- MACS Columns and MACS Separators: CD45<sup>+</sup> cells can be enriched by using MS or LS Columns. Positive selection 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
<b>Positive selection</b>			
MS	$10^7$	$2 \times 10^7$	MiniMACS, OctoMACS, SuperMACS II
LS	$4 \times 10^7$	$5 \times 10^7$	MidiMACS, QuadroMACS, SuperMACS II
	$4 \times 10^7$	$5 \times 10^7$	MultiMACS Cell24 Separator Plus
autoMACS	$5 \times 10^7$	$10^8$	autoMACS Pro
Multi-24 Column Block (per column)	$2 \times 10^7$	$2.5 \times 10^7$	MultiMACS Cell24 Separator Plus

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

▲ **Note:** If separating with LS Columns and the MultiMACS Cell24 Separator Plus use the Single-Column Adapter. Refer to the user manual for details.

- 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 REA (REAffinity™ antibodies: recombinantly engineered, lacking Fcγ-binding site) CD45 antibodies for flow cytometric analysis, e.g., CD45-PE-Vio® 770. For more information about antibodies refer to [www.miltenyibiotec.com/antibodies](http://www.miltenyibiotec.com/antibodies).
- ▲ **Note:** Due to expression of Fcγ receptors on tumor-infiltrating leukocytes REA antibodies are recommended.
- (Optional) Propidium Iodide Solution (# 130-093-233), DAPI Staining Solution (# 130-111-570), 7-AAD Staining Solution (# 130-111-568) or Viability™ Fixable Dyes (# 130-109-812, # 130-109-814, # 130-109-816) for flow cytometric exclusion of dead cells.
  - (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
  - (Optional) Pre-Separation Filters (30 μm) (# 130-041-407) to remove cell clumps.
  - (Optional) MACS SmartStrainers (30 μm) (# 130-098-458) to remove cell clumps.

## 2. Protocol

### 2.1 Sample preparation

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

For details refer to [www.gentlemacs.com/protocols](http://www.gentlemacs.com/protocols).

▲ Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, it is recommended to use density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).



### 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  $10^7$  total 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  $2 \times 10^7$  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 (MACS SmartStrainers (30 μm), # 130-098-458) 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 5 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 80 μL of buffer per  $10^7$  total cells.  
▲ **Note:** Always use freshly prepared buffer.
4. Add 20 μL of CD45 (TIL) MicroBeads per  $10^7$  total cells.
5. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
6. [(Optional) Add staining antibodies according to manufacturer's recommendation.
7. Add buffer to a final volume of 500 μL for up to  $5 \times 10^7$  cells.  
▲ **Note:** If more cells were used, split the sample onto multiple columns during magnetic separation.  
▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
8. 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 and the number of CD45<sup>+</sup> cells. For details refer to the table in section 1.4.

▲ **Note:** MS Columns are recommended for highest purity of CD45<sup>+</sup> cells. LS Columns are recommended for highest recovery of CD45<sup>+</sup> cells.

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic separation. 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.

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

#### Magnetic separation with MS or 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 the appropriate amount of buffer:  
MS: 500 μL      LS: 3 mL
  3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
  4. Wash column with the appropriate amount of buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 3.  
MS: 3×500 μL      LS: 2×1 mL
- ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.
  6. Pipette the appropriate amount of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.  
MS: 1 mL      LS: 3 mL

7. (Optional) To increase the purity of CD45<sup>+</sup> cells, the eluted fraction can be enriched over a second MS or LS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new 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.

### 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  $\geq 10$  °C.

▲ For appropriate resuspension volumes and cell concentrations, please visit [www.automacspro.com/autolabeling](http://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.
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:

##### Positive selection:

**Posseld2** for highest purity

or

**Possels** for highest recovery

Collect positive fraction in row C of the tube rack.

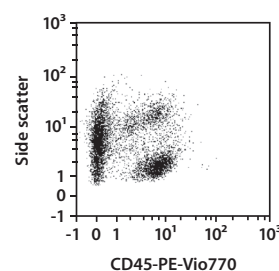
### 3. Example of a separation using CD45 (TIL)

#### MicroBeads

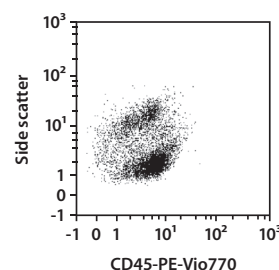
A human colorectal carcinoma sample was dissociated using the gentleMACS™ Octo Dissociator with Heaters in combination with the Tumor Dissociation Kit, human (# 130-095-929). CD45<sup>+</sup> TILs were isolated from the single-cell suspension using CD45 (TIL) MicroBeads, an LS Column, and a QuadroMACS™ Separator.

Cells were fluorescently stained with CD45-PE-Vio® 770 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.

#### A) Before separation



#### B) CD45<sup>+</sup> cells



Refer to [www.miltenyibiotec.com](http://www.miltenyibiotec.com) for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit [www.miltenyibiotec.com/local](http://www.miltenyibiotec.com/local) to find your nearest Miltenyi Biotec contact.

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