Miltenyi Biotec

CD8a$^+$ T Cell Isolation Kit
mouse

Order no. 130-104-075

Components

1 mL CD8a$^+$ T Cell Biotin-Antibody Cocktail, mouse: Cocktail of biotin-conjugated monoclonal antibodies against CD4, CD11b, CD11c, CD19, CD45R (B220), CD49b (DX5), CD105, MHC Class II, Ter-119, and TCRγ/δ.

2 mL Anti-Biotin MicroBeads: MicroBeads conjugated to monoclonal anti-biotin antibodies (isotype: mouse IgG1).

Capacity

For 10^9 total cells, up to 100 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.

Safety information

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

Before use, please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Cell separation methods

1.		**Fully automated cell labeling and separation using the autoMACS® Pro Separator**
2.		**Manual magnetic labeling**
2.1		**Subsequent automated cell separation using the autoMACS® Pro Separator**
2.2		**Subsequent semi-automated cell separation using the MultiMACS™ Cell24 Separator Plus**
2.3		**Subsequent manual cell separation**

General notes

▲ For an overview of the sample preparation procedure and recommendations for magnetic labeling and separation, refer to www.miltenyibiotec.com/faq.

▲ For product-specific background information and applications of this product, refer to the respective product page at www.miltenybiotec.com/130-104-075.

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). Degas buffer before use, as air bubbles could block the column.
- (Optional) Pre-Separation Filters, 30 µm (# 130-041-407) to remove cell clumps.
- Choose the appropriate MACS Separator and MACS Columns.

Column	Max. number of labeled cells	Max. number of total cells	Separator
LS	10^8	2×10^9	MidiMACS, QuadroMACS
autoMACS	2×10^8	4×10^9	autoMACS Pro
Multi-24 Column Block	10^8	10^9	MultiMACS Cell24 Separator Plus

▲ **Note:** If using the MultiMACS Cell24 Separator Plus with the Single-Column Adapter, please refer to the user manual for column capacities.

For additional requirements not included with the product, such as instruments or fluorochrome-conjugated antibodies, refer to www.miltenybiotec.com.

1.  Fully automated cell labeling and separation using the autoMACS® Pro Separator

▲ All buffer temperatures should be ≥10 °C.

▲ Place tubes in the following Chill Rack positions:

position A = sample, **position B** = negative fraction, **position C** = positive fraction.

1. For appropriate resuspension volumes and cell concentrations, please visit www.automacspro.com/autolabeling.
2. Turn on the instrument for automatic initialization.
3. Program autolabeling in the **Reagent** menu by selecting **Read Reagent** and scan the 2D barcode of each reagent vial with the barcode scanner on the autoMACS® Pro Separator. Place the reagent into the appropriate space on the reagent rack.
4. Place sample and collection tubes into the Chill Rack. Sample tube should be in row A, and the collection tubes in rows B and C.
5. Go to **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).
6. Enter sample volume into the **Volume** submenu.
7. Select **run**.

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

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

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2. Manual magnetic labeling

▲ Work fast, keep cells cold, and use pre-cooled solutions (2–8 °C).

▲ Volumes for magnetic labeling given below are for up to 10^7 total 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.

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

1. Prepare cells.
2. Resuspend cell pellet in 40 µL of buffer per 10^7 total cells.
3. Add 10 µL of Biotin-Antibody Cocktail per 10^7 total cells.
4. Mix well and incubate for 5 minutes in the refrigerator (2–8 °C).
5. Add 30 µL of buffer per 10^7 total cells.
6. Add 20 µL of Anti-Biotin MicroBeads per 10^7 total cells.
7. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
8. Proceed to magnetic separation (see 2.1, 2.2, or 2.3).

   ▲ **Note:** A minimum of 500 µL is required for magnetic separation. If necessary, add buffer to the cell suspension.

2.1 Subsequent automated cell separation using the autoMACS® Pro Separator

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

▲ Place tubes in the following Chill Rack positions:

position A = sample, **position B** = negative fraction, **position C** = positive fraction.

9. Prepare and prime the instrument.
10. Follow the instructions that are given in the user manual.
11. The program **"Depletes"** is recommended. Collect enriched $CD8a^+$ T cells at position B = negative fraction.

2.2 Subsequent semi-automated cell separation using the MultiMACS™ Cell24 Separator Plus

The MultiMACS™ Cell24 Separator Plus can be used with the Multi-24 Column Block or with up to nine LS, LD, or Whole Blood Columns in combination with the Single-Column Adapter.

9. Prepare and prime the instrument.
10. Follow instructions given on the Touch Screen Display and in the respective user manual.
11. The program **"DEPLETE"** is recommended. Collect enriched $CD8a^+$ T cells according to respective user manual.

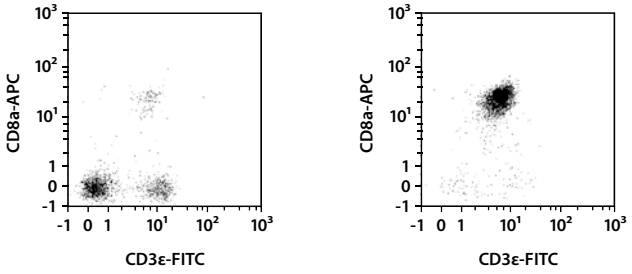
2.3 Subsequent manual cell separation

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

9. Place LS Column in the magnetic field of a suitable MACS Separator. For details refer to the respective MACS Column data sheet.
10. Prepare column by rinsing with 3 mL of buffer.
11. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells, representing the enriched $CD8a^+$ T cells.
12. Wash column with 3 mL of buffer. Collect unlabeled cells that pass through, representing the enriched $CD8a^+$ T cells, and combine with the effluent from step 11.
13. (Optional) Remove column from the separator and place it on a suitable collection tube. Pipette 5 mL of buffer onto the column. Immediately flush out the magnetically labeled non-$CD8a^+$ T cells by firmly pushing the plunger into the column.

Example of a separation using the $CD8a^+$ T Cell Isolation Kit

A single-cell suspension from mouse spleen was prepared using the program m_spleen_01.01 on the gentleMACS™ Dissociator. $CD8a^+$ T cells were isolated from this single-cell suspension using the $CD8a^+$ T Cell Isolation Kit, an LS Column, and a MidiMACS™ Separator. Cells were fluorescently stained with the MC CD8a T Cell Cocktail (# 130-092-915) 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.



For more information or assistance refer to our technical support.

Warranty

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