Miltenyi Biotec

CD8a (Ly-2) MicroBeads
mouse

FASTER PROTOCOL

Order no. 130-117-044

Components	2 mL CD8a (Ly-2) MicroBeads, mouse: MicroBeads conjugated to monoclonal anti-mouse CD8a (Ly-2) antibodies (isotype: rat IgG2a).
Capacity	For 2×10^9 total cells.
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 labels.

Safety information

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

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

Cell separation protocols

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

Alternatively:

Manual magnetic labeling

↓

Subsequent manual separation

or

↓

Subsequent automated cell separation using the autoMACS® Pro Separator

General notes

▲ For tips concerning sample preparation, magnetic labeling and separation, visit **www.miltenyibiotec.com/faq** and **www.miltenyibiotec.com/protocols**.

▲ For product-specific background information and applications of this product, refer to the respective product page at **www.miltenyibiotec.com/130-117-044**.

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
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS
LS	10^8	2×10^9	MidiMACS, QuadroMACS
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS Pro

Fully automated cell labeling and separation using 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.

▲ 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. Switch on the instrument for automatic initialization.
3. 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.
4. Place sample and collection tubes into the Chill Rack.
5. Go to the **Separation** menu and select the reagent name for each sample from the **Labeling** submenu (the correct labeling, separation (**Possels**), and wash protocols will be selected automatically for **positive selection** by default).

   ▲ **Note:** If depletion of target cells is desired, please select program **Depletes** from the menu.
6. Enter sample volume into the **Volume** submenu. Press **Enter**.
7. Select **Run**.
8. Collect enriched $CD8a^+$ T cell fraction at position C = positive fraction.
9. (Optional) Collect negative cell fraction at position B containing unlabeled cells that are depleted from $CD8a^+$ T cells.

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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 and determine cell number.
2. Resuspend cell pellet in 90 µL of buffer per 10^7 total cells.
3. Add 10 µL of CD8a (Ly-2) MicroBeads per 10^7 total cells.
4. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
5. Proceed to subsequent magnetic cell separation.

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

Subsequent manual cell separation

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

6. Place column in the magnetic field of a suitable MACS Separator. For details refer to the respective MACS Column data sheet.
7. Prepare column by rinsing with the appropriate amount of buffer:

   MS: 500 µL LS: 3 mL
8. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells, representing the $CD8a^-$ cell fraction.
9. Wash column with the appropriate amount of buffer. Collect unlabeled cells that pass through and combine with the effluent from step 8.

   MS: 2×500 µL LS: 1×3 mL
10. Remove column from the separator and place it on a suitable collection tube. Pipette the appropriate amount of buffer onto the column. Immediately flush out the magnetically labeled $CD8a^+$ T cells by firmly pushing the plunger into the column.

    MS: 1 mL LS: 5 mL
11. (Optional) To increase the purity of $CD8a^+$ T cells, the eluted fraction can be enriched over a second MS or LS Column. Repeat the magnetic separation procedure as described in steps 6 to 10 by using a new column.

Subsequent automated cell separation using 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.

▲ Place tubes in the following Chill Rack positions:

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

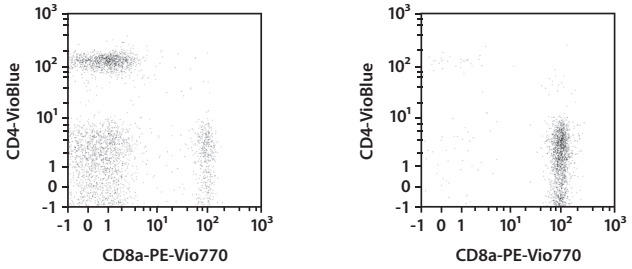
6. Prepare and prime the instrument.
7. Follow the instructions that are given in the user manual.
8. For a standard separation choose one of the following programs:

   Positive selection: Possels
   Collect positive fraction from row C of the tube rack.

   Depletion: Depletes
   Collect negative fraction in row B of the tube rack.

Example of a separation using CD8a (Ly-2) MicroBeads

A single-cell suspension from mouse spleen was prepared using the gentleMACS™ Dissociator. $CD8a^+$ T cells were isolated from this single-cell suspension using the CD8a (Ly-2) MicroBeads, an LS Column, and a QuadroMACS™ Separator. Cells were fluorescently stained with REAfinity™ Antibodies CD45-VioGreen™, CD4-VioBlue®, and CD8a-PE-Vio® 770 and analyzed by flow cytometry using the MACSQuant® Analyzer. Viable leukocytes were gated for analysis based on scatter signals, 7-AAD Staining Solution fluorescence, and CD45 expression.



For more information or assistance refer to our technical support.

Check out Miltenyi Biotec's flow cytometry solutions at **www.miltenyibiotec.com/MACSQuant** and explore the extensive antibody portfolio at **www.miltenyibiotec.com/antibodies.**

Warranty