Miltenyi Biotec

Direct Lineage Cell Depletion Kit
mouse

Order no. 130-110-470

Components	**1 mL Direct Lineage Cell Depletion Cocktail, mouse:** MicroBeads conjugated to monoclonal antibodies against CD5, CD11b, CD45R (B220), Anti-Gr-1 (Ly-6G/C), 7-4, and Ter-119.
Capacity	For 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 semi-automated cell separation using the MultiMACS™ Cell24 Separator Plus

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.miltenybiotec.com/130-110-470**.

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
LS or Multi-24 Column Block (per column)	10^8	10^9	MultiMACS Cell24 Separator Plus
autoMACS	2×10^8	4×10^9	autoMACS Pro

▲ **Note:** When using this kit the unwanted cell fraction is labeled and the target cells remain unlabeled. Depending on the target cell frequency, the labeled fraction can therefore represent the majority of the total cells.
To avoid blocking of the column, do not exceed the max. number of labeled cells per column. Estimate the number of labeled cells in the sample, split the sample if necessary and use the appropriate number of separation columns.

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

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, and wash protocols will be selected automatically).
6. Enter sample volume into the **Volume** submenu. Press **Enter**.
7. Select **Run**.
8. Collect enriched lineage-negative cell fraction at position B = negative fraction.

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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 2×10^7 total cells. Do not use less than 2×10^7 cells. 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 80 µL of buffer per 2×10^7 total cells.
3. Add 20 µL of Direct Lineage Cell Depletion Cocktail per 2×10^7 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 LS 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 3 mL of buffer.
8. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells, representing the enriched lineage-negative cells.
9. Wash column with 3×3 mL of buffer. Collect unlabeled cells that pass through, representing the enriched lineage-negative cells, and combine with the effluent from step 8.
10. (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 lineage-positive cells by firmly pushing the plunger into the column.

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 Columns in combination with the Single-Column Adapter.

6. Prepare and prime the instrument.
7. Follow instructions given on the Touch Screen Display and in the respective user manual.
8. The program **"DEPLETE"** is recommended. Collect enriched lineage-negative cells according to respective user manual.

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.

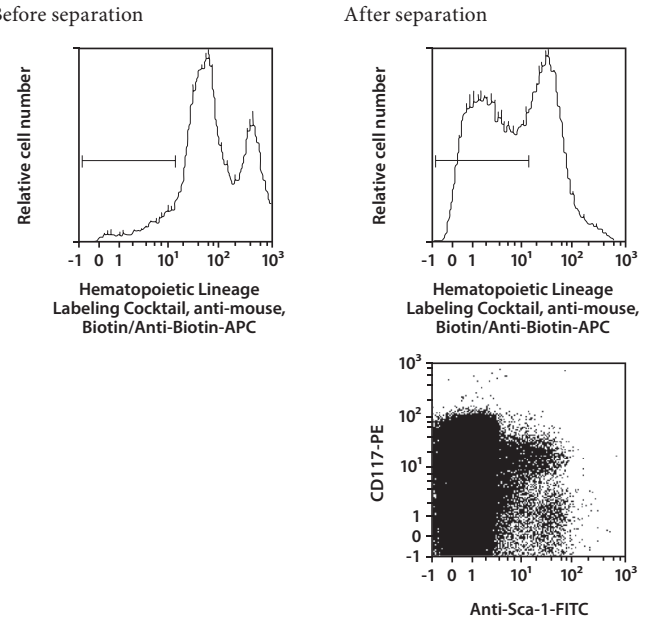
▲ Place tubes in the following Chill Rack positions:

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. The program **"Deplete2"** is recommended. Collect enriched lineage-negative cells at position B = negative fraction.

Example of a separation using the Direct Lineage Cell Depletion Kit

Isolation of untouched lineage-negative cells from a mouse bone marrow cell suspension using the Direct Lineage Cell Depletion Kit and a MidiMACS™ Separator with an LS Column. Cells were fluorescently stained with Hematopoietic Lineage Labeling Cocktail, anti-mouse, Biotin and Anti-Biotin-APC and analyzed by flow cytometry using the MACSQuant® Analyzer. To evaluate the LSK ($Lin^-Sca\text{-}1^+c\text{-}kit^+$) fraction, cells were further stained with CD117-PE (c-kit) and Anti-Sca-1-FITC. 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.

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Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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