Miltenyi Biotec

Pan B Cell Isolation Kit II
mouse

Order no. 130-104-443

Components	**1 mL FcR Blocking Reagent, mouse** **1 mL Pan B Cell Biotin-Antibody Cocktail, mouse:** Cocktail of biotin-conjugated monoclonal antibodies against CD3ε, CD4, CD8a, CD49b, Gr-1, and Ter119. **2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibodies (isotype: mouse IgG1).
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 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/130-104-443**.

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
MS	10^7	2×10^8	MiniMACS, OctoMACS
LS	10^8	2×10^9	MidiMACS, QuadroMACS
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.



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 B 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 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 35 µL of buffer per 10^7 total cells.
3. Add 5 µL of FcR Blocking Reagent per 10^7 total cells.
4. Add 10 µL of Biotin-Antibody Cocktail per 10^7 total cells.
5. Mix well and incubate for 5 minutes in the refrigerator (2–8 °C).
6. Add 30 µL of buffer per 10^7 total cells.
7. Add 20 µL of Anti-Biotin MicroBeads per 10^7 total cells.
8. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
9. 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.

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

    MS: 500 µL LS: 3 mL
12. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells, representing the enriched B cells.
13. Wash column with the appropriate amount of buffer. Collect unlabeled cells that pass through and combine with the effluent from step 11.

    MS: 500 µL LS: 3 mL
14. (Optional) 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 non-B cells by firmly pushing the plunger into the column.

    MS: 1 mL LS: 5 mL

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.

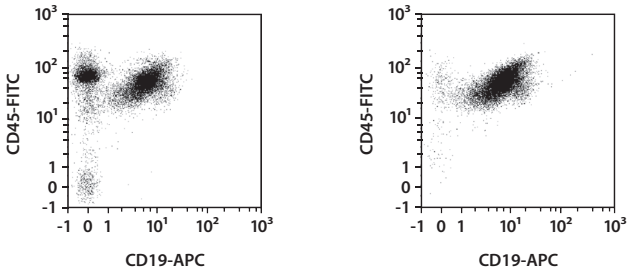
▲ Place tubes in the following Chill Rack positions:

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

10. Prepare and prime the instrument.
11. Follow the instructions that are given in the user manual.
12. The program **"Deplete"** is recommended. Collect enriched B cells at position B = negative fraction.

Example of a separation using the Pan B Cell Isolation Kit II

Pan B cells were isolated from spleen of a BALB/c mouse by using the Pan B Cell Isolation Kit II, an LS Column, and a MidiMACS™ Separator. The cells were fluorescently stained with CD19-APC and CD45-FITC 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.

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

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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