Miltenyi Biotec

Naive B Cell Isolation Kit II
human

Order no. 130-091-150

Components

1 mL Naive B Cell Biotin-Antibody Cocktail, human: Cocktail of biotin-conjugated monoclonal antibodies against CD2, CD14, CD16, CD27, CD36, CD43 and CD235a (Glycophorin A).

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.

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.

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/130-091-150**.

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 naive 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 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 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.

9. Place 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 the appropriate amount of buffer:

    MS: 500 µL LS: 3 mL
11. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells, representing the enriched naive B cells.
12. 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
13. (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 and non–naive 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.

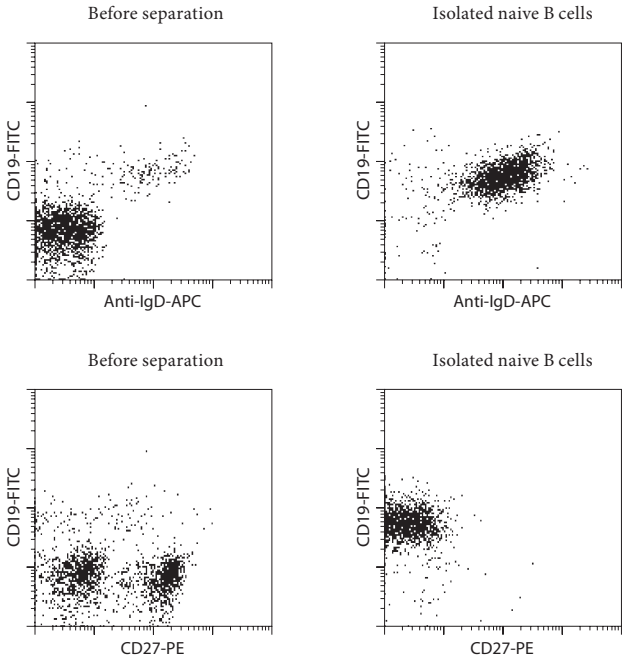
▲ Place tubes in the following Chill Rack positions:

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

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 naive B cells at position B = negative fraction.

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

Isolation of untouched naive B cells from human PBMCs using the Naive B Cell Isolation Kit II and an LS Column. The cells are fluorescently stained with CD19-FITC, CD27-PE, and Anti-IgD-APC. 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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