Miltenyi Biotec

Basophil Isolation Kit II
human

Order no. 130-092-662

Contents

1. Description

Components	**2 mL FcR Blocking Reagent:** human IgG. **2 mL Basophil Biotin-Antibody Cocktail:** Cocktail of biotin-conjugated monoclonal antibodies against CD3, CD4, CD7, CD14, CD15, CD16, CD36, CD45RA, HLA-DR and CD235a (Glycophorin A). **2×2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to a monoclonal anti-biotin antibody (mouse IgG1).
Capacity	For 2×10^9 total cells, up to 200 separations
Product format	The Biotin-Antibody Cocktail and the Anti-Biotin MicroBeads 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.

1.1 Principle of the MACS® Separation

Using the Basophil Isolation Kit II, human, basophils are isolated by depletion of non-basophils (negative selection). Non-basophils are indirectly magnetically labeled with a cocktail of biotin-conjugated monoclonal antibodies, as primary labeling reagent, and anti-biotin monoclonal antibodies conjugated to MicroBeads, as secondary labeling reagent. In between the two labeling steps no washing steps are required. The magnetically labeled non-basophils are depleted by retaining them on a MACS® Column in the magnetic field of a MACS Separator, while the unlabeled basophils pass through the column.

1.2 Background information

The Basophil Isolation Kit II is an indirect magnetic labeling system for the isolation of untouched basophils from human peripheral blood mononuclear cells (PBMCs). Non-basophils, i.e. T cells, NK cells, B cells, monocytes, dendritic cells, erythroid cells, platelets, neutrophils, and eosinophils are indirectly magnetically labeled using a cocktail of biotin-conjugated antibodies against CD3, CD4, CD7, CD14, CD15, CD16, CD36, CD45RA, HLA-DR and CD235a (Glycophorin A), and Anti-Biotin MicroBeads. Isolation of highly pure unlabeled basophils is achieved by depletion of the magnetically labeled cells.

1.3 Applications

- Functional studies on basophils where effects due to antibody-crosslinking of cell surface proteins should be avoided.
- Studies on signal requirements for basophil activation, induction of mediator synthesis and release etc.
- Studies on the involvement of basophils in hypersensitivity reactions.
- Studies on surface markers expressed by basophils.
- Studies on cytokine expression by basophils.

1.4 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). Keep buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column.

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: Choose the appropriate MACS Separator and MACS Columns according to the number of labeled cells and to the number of total cells.

Column	Max. number of labeled cells	Max. number of total cells	Separator
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
XS	10^9	2×10^{10}	SuperMACS
autoMACS	2×10^8	4×10^9	autoMACS Pro, autoMACS

▲ **Note:** Column adapters are required to insert certain columns into the VarioMACS™ or SuperMACS™ Separators. For details see the respective MACS Separator data sheet.

- (Optional) Fluorochrome-conjugated antibody for flow cytometric analysis, e.g. CD203c antibodies, CD123-FITC (# 130-090-897), CD123-PE (# 130-090-899), CD123-APC (# 130-090-901), CD303 (BDCA-2)-FITC (# 130-090-510), CD303 (BDCA-2)-PE (# 130-090-511), CD303 (BDCA-2)-APC (# 130-090-905), CD45-FITC (# 130-080-202), CD45-PE (# 130-080-201), or CD45-APC (# 130-091-230). For more

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information about fluorochrome-conjugated antibodies see www.miltenyibiotec.com.

- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation, for example, using Ficoll-Paque™.

▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully aspirate supernatant. Repeat washing step.

When working with tissues or lysed blood, prepare a single-cell suspension using standard methods.

For details see the protocols section at www.miltenyibiotec.com/protocols.

▲ Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, we recommend using density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).

2.2 Magnetic labeling

▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ Volumes for magnetic labeling given below are for up to 10^7 total cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic labeling. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

▲ The recommended incubation temperature is 2–8 °C. Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 30 µL of buffer per 10^7 total cells.
4. Add 10 µL of FcR Blocking Reagent per 10^7 total cells.
5. Add 10 µL of Basophil Biotin-Antibody Cocktail per 10^7 total cells.
6. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
7. Add 30 µL of buffer per 10^7 total cells.
8. Add 20 µL of Anti-Biotin MicroBeads per 10^7 total cells.
9. Mix well and incubate for an additional 15 minutes in the refrigerator (2–8 °C).
10. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
11. Resuspend up to 10^8 cells in 500 µL of buffer.
    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
12. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of total cells. For details see table in section 1.4.

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

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details see the respective MACS Column data sheet.
2. Prepare column by rinsing with the appropriate amount of buffer:
   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells, representing the enriched basophil fraction.
4. Wash column with the appropriate amount of buffer. Collect unlabeled cells that pass through and combine with the effluent from step 3. This fraction represents the enriched basophils.
   MS: 3×500 µL LS: 3×3 mL
   ▲ **Note:** Perform washing steps by adding buffer aliquots only when the column reservoir is empty.
5. (Optional) Elute retained cells outside of the magnetic field. This fraction represents the magnetically labeled non-basophils.

Magnetic separation with XS Columns

For instructions on the column assembly and the separation refer to the XS Column data sheet.

Magnetic separation with the autoMACS™ Pro Separator or the autoMACS™ Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS™ Pro Separator or the autoMACS Separator.

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

▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual.

Magnetic separation with the autoMACS™ Pro Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place

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sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.

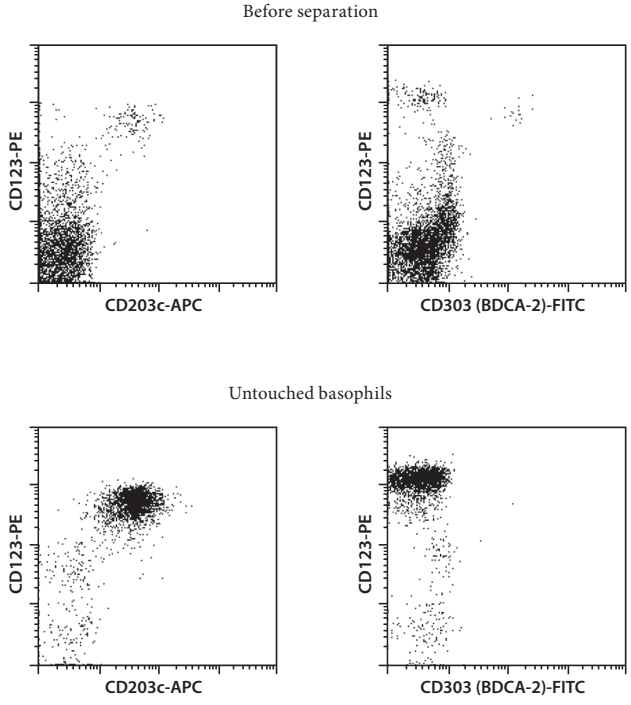
3. Choose the following program: "Depletes"
   Collect negative fraction in row B of the tube rack. This fraction represents the enriched basophils.
4. (Optional) Collect positive fraction in row C. This fraction represents the magnetically labeled non-basophils.

Magnetic separation with the autoMACS™ Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos1.
3. Choose the following program: "Depletes"
   Collect negative fraction from outlet port neg1. This fraction represents the enriched basophils.
4. (Optional) Collect positive fraction from outlet port pos1. This fraction represents the magnetically labeled non-basophils.

3. Example of a separation using the Basophil Isolation Kit II

Isolation of untouched basophils from PBMCs using the Basophil Isolation Kit II and an LS Column. Cells are fluorescently stained with CD203c-APC, CD123-PE, and CD303 (BDCA-2)-FITC for the exclusion of plasmacytoid dendritic cells. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.



4. Reference

1. Dzionek, A. *et al.* (2000) BDCA-2, BDCA-3, and BDCA-4: Three markers for distinct subsets of dendritic cells in human peripheral blood. J. Immunol. 165: 6037–6046.

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.

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

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