Miltenyi Biotec

Diamond Basophil Isolation Kit
human

Order no. 130-094-434

Contents

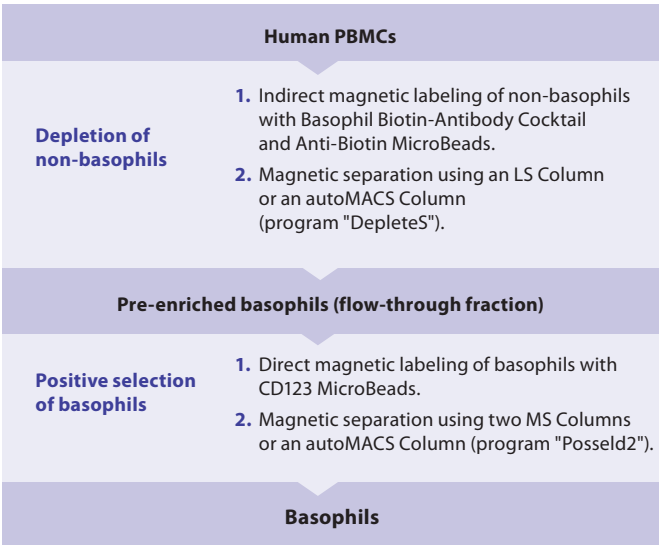
1. Description
   - 1.1 Principle of the MACS® Separation
   - 1.2 Background information
   - 1.3 Applications
   - 1.4 Reagent and instrument requirements
2. Protocol
   - 2.1 Sample preparation
   - 2.2 Magnetic labeling of non-basophils
   - 2.3 Magnetic separation: Depletion of non-basophils
   - 2.4 Magnetic labeling of pre-enriched basophils
   - 2.5 Magnetic separation: Positive selection of basophils
3. Example of a separation using the Diamond Basophil Isolation Kit
4. References

1. Description

Components	**2 mL Basophil Biotin-Antibody Cocktail, human:** 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 (isotype: mouse IgG1). **2 mL CD123 MicroBeads, human:** MicroBeads conjugated to monoclonal CD123 antibodies (isotype: mouse IgG2a). **2 mL FcR Blocking Reagent, human:** isotype: human IgG.
Capacity	For 2×10^9 total cells.
Product format	The Basophil Biotin-Antibody Cocktail, the Anti-Biotin MicroBeads, the CD123 MicroBeads, and the FcR Blocking Reagent 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

The Diamond Basophil Isolation Kit is developed for the isolation of basophilic granulocytes from peripheral blood mononuclear cells (PBMCs). Isolation of basophils is performed in a two-step procedure. First, non-basophil cells are indirectly magnetically labeled with a cocktail of biotin-conjugated antibodies and Anti-Biotin MicroBeads. Upon subsequent magnetic separation of the cells over a MACS® Column that is placed in a magnetic field of a MACS Separator, the magnetically labeled non-basophils are retained on the column while the unlabled basophils pass through. In the second step, the enriched basophils are directly labeled with CD123 MicroBeads. Upon subsequent magnetic separation, the basophils are eluted after removal of the column from the magnetic field. The expected purity of isolated basophils after separation is close to 100%.



1.2 Background information

Basophilic granulocytes, also known as basophils, are a rare population of leukocytes with frequencies below 1% in peripheral blood. First described by Paul Ehrlich in 1879, basophils have long been considered as circulating mast cells. They express high affinity immunoglobulin (Ig) E receptors (FcεRI). Upon receptor cross-linking by binding of antigen-IgE complexes, basophils degranulate and thereby release inflammatory mediators such as histamine and leukotriene. Basophils play thus a major role in allergy, asthma, and immunity.[1–4]

1.3 Applications

- 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 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

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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: Basophils can be enriched by using MS or LS Columns. Depletion and subsequent positive selection can also be performed by using the autoMACS Pro or the autoMACS Separator.

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
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 antibodies for flow cytometric analysis, e.g., CD123-PE (# 130-090-899), CD203c-APC (# 130-092-344), and CD303 (BDCA-2)-FITC (# 130-090-510). For more 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

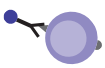
When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation, for example, using Ficoll-Paque™.

▲ **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 tissue 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 of non-basophils

▲ 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 10^8 total cells. When working with fewer than 10^8 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 2×10^8 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 300 µL of buffer per 10^8 total cells.
4. Add 100 µL of FcR Blocking Reagent per 10^8 total cells.
5. Add 100 µL of Basophil Biotin-Antibody Cocktail per 10^8 total cells.
6. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
7. Add 300 µL of buffer per 10^8 total cells.
8. Add 200 µL of Anti-Biotin MicroBeads per 10^8 total cells.
9. Mix well and incubate for an additional 15 minutes in the refrigerator (2–8 °C).
10. Wash cells by adding 10–20 mL of buffer per 10^8 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: Depletion of non-basophils

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of basophils. 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 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 3 mL of buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells, representing the enriched basophil fraction.
4. Wash column by adding 3 mL of buffer. Collect unlabeled cells that pass through and combine with the effluent from step 3. This fraction represents the enriched basophils.
   ▲ **Note:** Perform washing steps by adding buffer aliquots only when the column reservoir is empty.

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5. (Optional) Elute retained cells outside of the magnetic field. This fraction represents the magnetically labeled non-basophils.
6. Proceed to magnetic labeling (2.4) with the enriched basophils.

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 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.
5. Proceed to magnetic labeling (2.4) with the enriched 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.
5. Proceed to magnetic labeling (2.4) with the enriched basophils.



2.4 Magnetic labeling of pre-enriched basophils

▲ 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 an initial starting cell number of up to 10^8 total cells. When working with fewer than 10^8 initial cells, use the same volumes as indicated. When working with higher initial cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^8 total initial 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. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet directly in 100 µL CD123 MicroBeads.
3. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
4. Wash cells by adding 1–2 mL of buffer per 10^8 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
5. Resuspend up to 10^8 cells in 500 µL of buffer.
   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
6. Proceed to magnetic separation (2.5).



2.5 Magnetic separation: Positive selection of basophils

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of basophils. 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 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 with500 µL of buffer:
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column by adding 3×500 µL of buffer. Collect unlabeled cells that pass through and combine with the effluent from step 3.
   ▲ **Note:** Perform washing steps by adding buffer aliquots only when the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.
   ▲ **Note:** To perform a second column run, you may elute the cells directly from the first onto the second, equilibrated column instead of a collection tube.
6. Pipette 1 mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
7. To increase the purity of basophils, the eluted fraction can be enriched over a second MS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

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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 sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For a standard separation choose one of the following programs:

   Positive selection: "Posseld2"
   Collect positive fraction in row C of the tube rack.

Magnetic separation with the autoMACS™ Separator

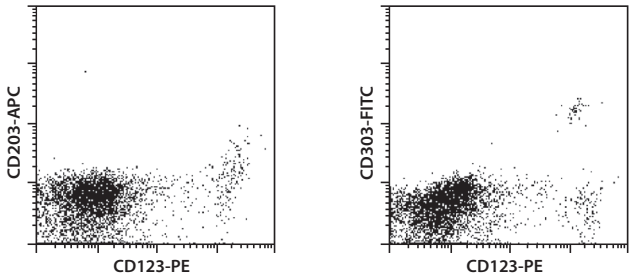
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos2.
3. For a standard separation choose one of the following programs:

   Positive selection: "Posseld2"
   Collect positive fraction from outlet port pos2.

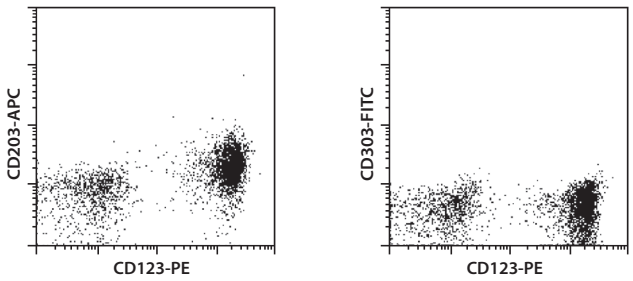
3. Example of a separation using the Diamond Basophil Isolation Kit

Basophils were isolated from human PBMCs using the Diamond Basophil Isolation Kit and two MS Columns. Cells were fluorescently stained with CD123-PE (# 130-090-899), CD203c-APC (# 130-092-344), and CD303 (BDCA-2)-FITC (# 130-090-510) and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

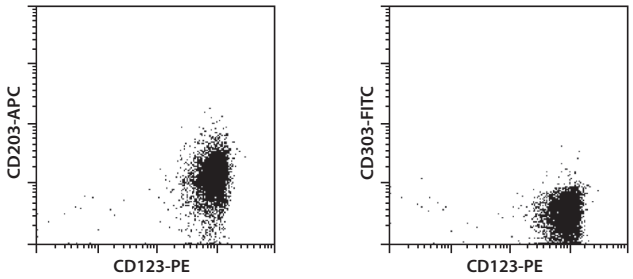
PBMCs before separation



Pre-enriched basophils



Isolated basophils



4. References

1. Shiratori, I. *et al.* (2005) Down-Regulation of Basophil Function by Human CD200 and Human Herpesvirus-8 CD200. J. Immunol. 175: 4441–4449.
2. Sloane, D.E. *et al.* (2004) Leukocyte immunoglobulin-like receptors: novel innate receptors for human basophil activation and inhibition. Blood 104: 2832–2839.
3. Falcone F.H. *et al.* (2000) The human basophil: a new appreciation of its role in immune responses. Blood 96: 4028–4038.
4. Karasuyama, H. *et al.* (2009) Newly discovered roles for basophils: a neglected minority gains new respect. Nature reviews 9: 9–13.

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.

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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