Miltenyi Biotec

Eosinophil Isolation Kit
human

Order no. 130-092-010

Contents

1. Description

Components	**1 mL Eosinophil Biotin-Antibody Cocktail, human:** Cocktail of biotin-conjugated monoclonal antibodies against CD2, CD14, CD16, CD19, CD56, CD123, 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, up to 100 separations.
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 label.

1.1 Principle of MACS® Separation

Using the Eosinophil Isolation Kit, human eosinophils are isolated by depletion of non-eosinophils (untouched isolation). Non-eosinophils 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-eosinophils are depleted by retaining them on a MACS® Column in the magnetic field of a Separator, while the unlabeled eosinophils pass through the column.

1.2 Background information

Eosinophils are involved in innate as well as adaptive immunity. They exert functions in the immunological response to various pathological conditions, e.g., parasitic infections, asthma, and gastrointestinal disorders. Eosinophils are characterized by granules that contain several cytotoxic proteins (e.g. major basic protein, MBP) and ribonucleases. Eosinophils act through release of these pre-formed molecules as well as various cytokines, chemokines, lipid mediators, and neuromodulators. They are activated in response to a number of stimuli, such as tissue injury, infections, allergens, and tumors. Eosinophils interact with various other immune cells, for example, they serve as antigen-presenting cells and thus can activate T cells. They also possess the capacity to activate mast cells by releasing MBP.[1]

1.3 Applications

- Functional studies on eosinophils.
- Studies on signal transduction in eosinophils.
- Studies on eosinophil activation mechanisms.
- Studies on cytokine production by eosinophils.

1.4 Reagent and instrument requirement

- 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 in 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
Depletion			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
XS	10^9	2×10^{10}	SuperMACS II
autoMACS	2×10^8	4×10^9	autoMACS Pro, autoMACS

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

- Erythrocyte lysis solution, e.g., Red Blood Cell Lysis Solution (10×) (# 130-094-183).
- Ficoll-Paque™.
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD16-FITC (# 130-091-244), CD16-PE (# 130-091-245), CD16-APC (# 130-091-246), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856). For more information about other fluorochrome conjugates refer to www. miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.

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- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
- (Optional) Pre-Separation Filters, 30 µm (# 130-041-407) to remove cell clumps.

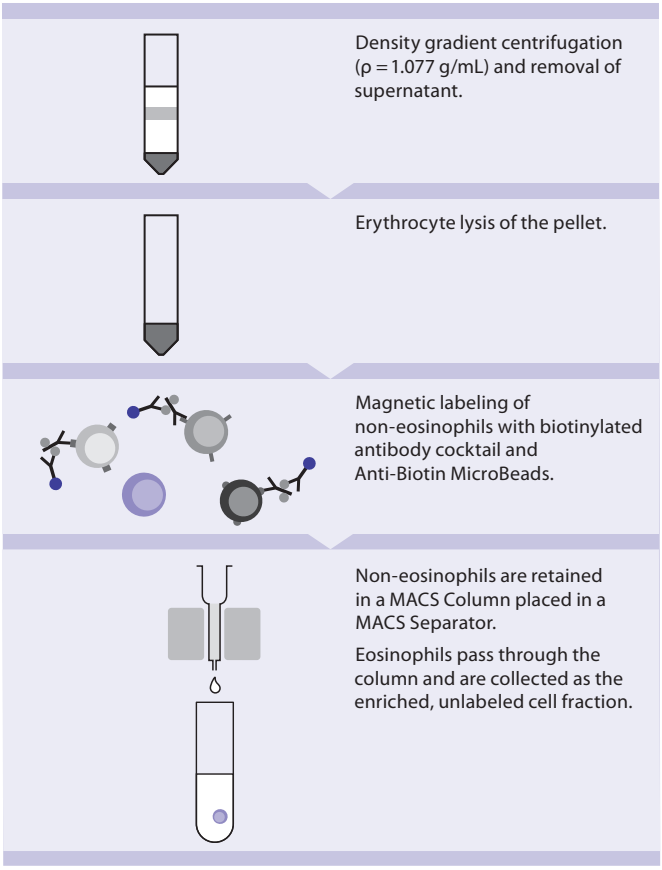
2. Protocol

2.1 Sample preparation

1. Start with fresh human blood treated with an anticoagulant, e.g. heparin, citrate, ACD-A, or citrate phosphate dextrose (CPD), or leukocyte-rich buffy coat not older than 8 hours.
2. Dilute cells by adding one volume of PBS containing 2 mM EDTA or 0.6% ACD-A.
3. Carefully layer 20 mL of diluted cell suspension over 20 mL Ficoll-Paque (ρ = 1.077 g/mL) in a 50 mL conical tube and centrifuge at 600×g for 30 minutes at 20 °C in a swinging bucket rotor without brake.
4. Carefully remove and discard the plasma, the mononuclear cells which form a layer at the Plasma:Ficoll interface and the Ficoll-Paque, leaving the red cell pellet undisturbed.
5. Resuspend the red cell pellet in 1× Red Blood Cell Lysis Solution and fill the complete 50 mL conical tube with lysis solution.
6. Incubate for 10 minutes at room temperature (19–25 °C).
7. Centrifuge at 300×g for 8 minutes at 20 °C. Carefully remove supernatant completely
8. Wash cells by adding 50 mL of buffer.

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

Principle of sample preparation



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 separation. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, 30 µm, # 130-041-407) to remove cell clumps which may clog the column. Wet filter with buffer before use.

▲ Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice may require increased incubation times.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 40 µL of buffer per 10^7 total cells.
4. Add 10 µL of Biotin-Antibody Cocktail per 10^7 total cells.
5. Mix well and incubate for 10 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 an additional 15 minutes in the refrigerator (2–8 °C).
9. Wash cells with buffer by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
10. Resuspend up to 10^8 cells in 500 µL of buffer.
    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
11. Proceed to magnetic separation.

2.3 Magnetic separation

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

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details refer to 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.

4. Collect unlabeled cells that pass through and wash column with the appropriate amount of buffer. Collect total effluent; this is the unlabeled cell fraction representing the enriched eosinophil fraction. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
   MS: 3×500 µL LS: 3×3 mL
5. (Optional) Elute retained cells outside of the magnetic field. This fraction represents the magnetically labeled non-eosinophils.

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 sample tube in row A of the tube rack and fraction collection tubes in rows B and C.
3. For a standard separation choose the following program:
   Depletion: Depletes
   Collect negative fraction in row B 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 pos1.
3. For a standard separation choose the following program:
   Depletion: Depletes
   Collect negative fraction from outlet port neg1.

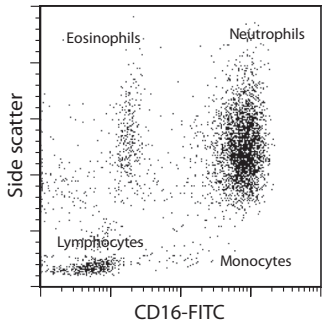
2.4 (Optional) Evaluation of eosinophil purity

The purity of the enriched eosinophils can be evaluated by flow cytometry or by microscopy. Stain aliquots of the cell fractions with a fluorochrome-conjugated antibody, e.g., CD16-FITC (# 130-091-244), as recommended in the respective data sheets. Eosinophils are CD16 negative and SSC^{high}.
Labeling of non-eosinophils with the Biotin-Antibody Cocktail can be visualized by counterstaining with a fluorochrome-conjugated anti-biotin antibody, e.g., Anti-Biotin-PE (# 130-090-756) or Anti-Biotin-APC (# 130-090-856). Staining with fluorochrome-conjugated streptavidin is not recommended.

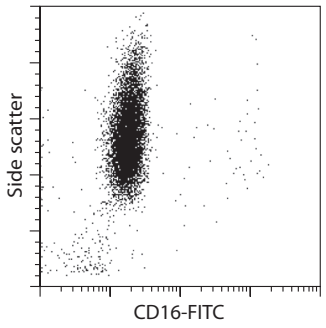
3. Example of a separation using the Eosinophil Isolation Kit

Untouched eosinophils were isolated from human peripheral blood by using the Eosinophil Isolation Kit, an LS Column, and an appropriate MACS Separator. Cells are fluorescently stained with CD16-FITC (# 130-091-244). Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

Before separation



Isolated untouched eosinophils



4. Reference

1. Rothenberg, M.E. and Hogan, S.P. (2006) The eosinophil. Annu. Rev. Immunol. 24: 147–174.

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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