Miltenyi Biotec

ILC2 Isolation Kit
human

Order no. 130-114-825

Contents

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.

1. Description

This product is for research use only.

Components

2×1 mL Biotin-Antibody Cocktail, human: Cocktail of biotin-conjugated monoclonal antibodies against CD2, CD3, CD11b, CD14, CD15, CD16, CD19, CD56, CD123, and CD235a (Glycophorin A).

2×2 mL Anti-Biotin MicroBeads: MicroBeads conjugated to monoclonal anti-biotin antibody (isotype: mouse IgG1).

2 mL FcR Blocking Reagent, human: human IgG.

200 µL CD294 (CRTH2)-PE, human: Monoclonal anti-human CD294 (CRTH2) antibody conjugated to R-phycoerythrin (PE) (isotype: rat IgG2a).

2 mL Anti-PE MicroBeads: MicroBeads conjugated to monoclonal anti-PE antibody (isotype: mouse IgG1).

Capacity For 2×10^9 total cells, up to 200 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 the MACS® Separation

The isolation of human type 2 innate lymphoid cells (ILC2) is performed in a two-step procedure. First, the lineage-positive cells are indirectly magnetically labeled with a cocktail of biotin-conjugated antibodies, as primary labeling reagent, and anti-biotin monoclonal antibodies conjugated to MicroBeads, as secondary labeling reagent. The labeled cells are subsequently depleted by separation over two MACS® Columns, which are placed in the magnetic field of a MACS Separator. The magnetically labeled lineage-positive cells are retained in the columns, while the unlabeled lineage-negative cells run through.

In the second step, the lineage-negative cells are labeled with CD294 (CRTH2)-PE, as primary labeling reagent, and Anti-PE MicroBeads, as secondary labeling reagent and isolated by positive selection from the pre-enriched lineage-negative cell fraction by separation over a MACS Column, which is placed in the magnetic field of a MACS Separator.

After removing the column from the magnetic field, the magnetically retained CD294 (CRTH2)$^+$ ILC2 can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the CD294 (CRTH2)$^+$ ILC2 must be separated over a second column.

Periperhal blood mononuclear cells: Depletion of lineage-positive cells

1. Indirect magnetic labeling of lineage-positive cells with Biotin-Antibody Cocktail and Anti-Biotin MicroBeads.
2. Magnetic separation using two LS Columns.

Pre-enriched lineage-negative cells (flow-through fraction): Positive selection of CD294 (CRTH2)$^+$ ILC2

1. Indirect magnetic labeling of CD294 (CRTH2)$^+$ cells with CD294 (CRTH2)-PE and Anti-PE MicroBeads.
2. Magnetic separation using two MS Columns.

CD294 (CRTH2)$^+$ ILC2 (eluted fraction)
CD294 (CRTH2)$^-$ cells (flow-through fraction)

1.2 Background information

Human type 2 innate lymphoid cells (ILC2) are defined as lineage (CD2, CD3, CD14, CD16, CD19, CD56, CD235a, CD123)-negative and (CD127, CD161, CRTH2)-positive lymphocytes.[1]

Using the ILC2 Isolation Kit, human type 2 innate lymphoid cells are pre-enriched in a first step by depletion of lineage-positive cells, to facilitate the enrichment of high pure target cells through

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following enrichment using CD294 (CRTH2)-PE and Anti-PE MicroBeads.

1.3 Applications

- Isolation of human type 2 innate lymphoid cells (ILC2) from human peripheral blood mononuclear cells (PBMCs) for further phenotypical or functional characterization.

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: Depletion of lineage-positive cells can be performed on two LS Columns. The subsequent positive selection of CD294 $(CRTH2)^+$ ILC2 can be performed on two MS Columns.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Depletion			
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS II

  ▲ **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.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD161-PE-Vio® 770 (clone: REA631), CD127-APC (clone: REA614), CD45-VioBlue® (clone REA747). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filters (30 µm) (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

When working with anticoagulated peripheral blood or 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.

For details refer to the protocols section at www.miltenyibiotec.com/protocols.

2.2 Magnetic labeling of lineage-positive cells

▲ 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 (30 µm), # 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. 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. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
8. Add 20 µL of Anti-Biotin MicroBeads per 10^7 cells.
9. Mix well and incubate for 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).

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2.3 Magnetic separation: Depletion of lineage-positive cells

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

▲ To achieve highest purities, perform two consecutive column runs.

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

Depletion with LS Columns

1. Place LS Column in the magnetic field of a suitable MACS Separator. For details refer to LS 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.
4. Wash column with 3×3 mL of buffer. Collect total effluent and combine with the effluent from step 3; this is the unlabeled pre-enriched CD294 (CRTH2)$^+$ ILC2 fraction.

   ▲ **Note:** Perform washing steps by adding buffer aliquots only when the column reservoir is empty.
5. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
6. Resuspend up to 10^8 cells in 500 µL of buffer.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
7. Repeat the magnetic separation procedure as described in steps 1 to 4 by using a new LS Column.
8. Proceed to 2.4 for further isolation of CD294 (CRTH2)$^+$ ILC2.

2.4 Magnetic labeling of CD294 (CRTH2)$^+$ cells

▲ Volumes for magnetic labeling given below are for an initial starting cell number of up to 10^7 total cells. For higher initial cell numbers, scale up all volumes accordingly.

1. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 78 µL of buffer per 10^7 total cells.
3. Add 20 µL of FcR Blocking Reagent and 2 µL of CD294 (CRTH2)-PE per 10^7 total cells.
4. Mix well and incubate for **20 minutes in the dark at room temperature (20–25 °C)**.
5. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
6. Resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
7. Add 20 µL of Anti-PE MicroBeads per 10^7 total cells.
8. Mix well and incubate for an additional 15 minutes in the dark in the refrigerator (2–8 °C).
9. Wash cells 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 (2.5).

2.5 Magnetic separation: Positive selection of CD294 (CRTH2)$^+$ ILC2

Positive selection with MS Columns

▲ To achieve highest purities, perform two consecutive column runs.

1. Place MS Column in the magnetic field of a suitable MACS Separator. For details refer to MS Column data sheet.
2. Prepare column by rinsing with 500 µL of buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with 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 as soon as 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 purity of CD294 (CRTH2)$^+$ ILC2, the eluted fraction must 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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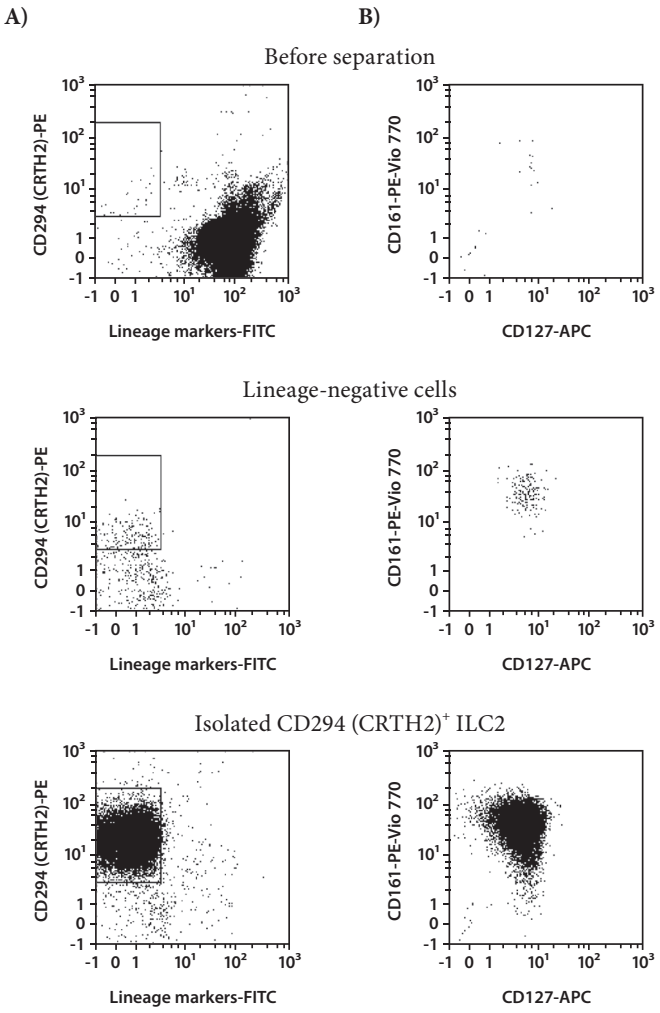
3. Example of a separation using the ILC2 Isolation Kit

Type 2 innate lymphoid cells (ILC2) were isolated from peripheral blood mononuclear cells by using the ILC2 Isolation Kit, two LS and two MS Columns, as well as a MidiMACS™ Separator and a MiniMACS™ Separator. The cells were fluorescently stained with CD45-VioBlue® and lineage markers (CD2, CD3, CD14, CD16, CD123, CD235a (Glycophorin A) conjugated to FITC, CD19 and CD56 conjugated to Vio Bright FITC), and CD294 (CRTH2)-PE (A) or CD161-PE-Vio® 770 and CD127-APC (B) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from analysis based on scatter signals, CD45, and propidium iodide fluorescence.



4. References

1. Mjösberg, J. M. *et al.* (2011) Human IL-25– and IL-33–responsive type 2 innate lymphoid cells are defined by expression of CRTH2 and CD161. Nat. Immunol. 12: 1055–1062.

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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