Miltenyi Biotec

Plasma Cell Isolation Kit II
human

Order no. 130-093-628

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 mL Non-Plasma Cell Biotin-Antibody Cocktail, human:
Cocktail of biotin-conjugated monoclonal antibodies against CD2, CD3, CD10, CD13, CD15, CD22, CD34, CD123, and CD235a (Glycophorin A).

2×2 mL Non-Plasma Cell MicroBead Cocktail, human:
Cocktail of CD14 MicroBeads and Anti-Biotin MicroBeads.

2 mL CD56 MicroBeads, human:
MicroBeads conjugated to monoclonal anti-human CD56 antibodies (isotype: mouse IgG1).

1 mL CD38 MicroBeads, human:
MicroBeads conjugated to monoclonal anti-human CD38 antibodies (isotype: mouse IgG2a).

Capacity
For 2×10^9 total cells, up to 20 separations.

Product format
All reagents 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 Plasma Cell Isolation Kit II has been developed for the isolation of CD38$^+$ plasma cells from peripheral blood mononuclear cells (PBMCs) and bone marrow by performing two subsequent magnetic separation steps. First, non-plasma cells are indirectly magnetically labeled with a cocktail of biotin-conjugated monoclonal antibodies and anti-Biotin MicroBeads. The labeled cells are subsequently depleted by separation over a MACS® Column. In the second step, CD38$^+$ plasma cells are directly labeled with CD38 MicroBeads and isolated by positive selection from the pre-enriched plasma cell fraction. After removing the column from the magnetic field, the magnetically retained CD38$^+$ plasma cells can be eluted as the positively selected cell fraction.

Human PBMCs or bone marrow: Depletion of non-plasma cells

1. Indirect magnetic labeling of non-target cells with the Non-Plasma Cell Biotin-Antibody Cocktail, CD56 MicroBeads, and the Non-Plasma Cell MicroBead Cocktail.
2. Magnetic separation using an LD Column or an autoMACS Column (program "Depl025").

Pre-enriched CD38$^+$ plasma cells (flow-through fraction):

1. Direct magnetic labeling of CD38$^+$ cells with CD38 MicroBeads.
2. Magnetic separation using two MS Column or an autoMACS Column (program "Posselsd2).

CD38$^+$ plasma cells

1.2 Background information

Human plasma cells are a heterogenous cell population comprising several subsets from short-lived and proliferative plasmablasts in lymphoid tissues, transitional plasma cells in peripheral blood to long-lived and non-dividing plasma cells in bone marrow[1-3]. Plasma cells of all differentiation stages are identified by the expression of high levels of the CD38 antigen. Other surface markers, however, are differentially regulated dependent on the stage of differentiation and the spatial localization. Plasma cells in the blood circulation lack the expression of the typical B cell marker CD22 and were found to express lower levels of CD19 than mature B cells.[1] They are further characterized as being CD27^{++}, CD31$^+$, CD44$^+$, CD45$^+$, CD56$^-$, CD62L$^+$, CD86$^+$, and HLA-DR$^+$.[1,4,6] A subset of CD38$^+$ blood plasma cells further expresses the CD138 antigen, whereas all CD38$^+$ plasma cells are also CD138$^+$ in bone marrow.[4] For the isolation of malignant plasma cells from bone marrow, the addition of CD56 MicroBeads may be left aside to avoid depletion of CD56-expressing tumor cells.

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1.3 Applications

Isolation of plasma cells from PBMCs or bone marrow for:

- Analysis of signal transduction pathways.
- Molecular analysis, for example, gene expression profiling.
- Studies on plasma cell dysfunctions, for example, in allergy, asthma, autoimmunity, or infectious diseases.
- Studies on the migrational behaviour of plasma cells.

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. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: Depletion of non-plasma cells is performed on an LD Column. The subsequent positive selection of $CD38^+$ plasma cells is performed on two MS Columns. Depletion and positive selection can also be performed by using the autoMACS Pro Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
Depletion and positive selection			
autoMACS	2×10^8	4×10^9	autoMACS Pro

▲ **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 evaluation of plasma cells, e.g., CD38-FITC (# 130-092-259), CD19-PE (# 130-091-247), CD19-APC (# 130-091-248), CD138-PE (# 130-081-301), and CD138-APC (# 130-091-250). For more information about other fluorochrome conjugates see www.miltenyibiotec.com/antibodies.
- (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, 30 µm (# 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 refer to 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-plasma 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 10^8 total cells. When working with higher or fewer (minimum 10^7 total cells) cell numbers, scale up or down 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, 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 400 µL of buffer per 10^8 total cells.
4. Add 100 µL of Non-Plasma Cell Biotin-Antibody Cocktail per 10^8 total cells.
5. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
6. Add 200 µL of buffer per 10^8 total cells.
7. Add 200 µL of Non-Plasma Cell MicroBead Cocktail per 10^8 total cells.
8. Add 100 µL of CD56 MicroBeads per 10^8 total cells.
9. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
10. Wash cells by adding 5–10 mL of buffer per 10^8 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

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11. Resuspend cell pellet in buffer:
    Depletion with LD Column: 500 µL for up to 1.25×10^8 cells.
    Depletion with autoMACS Pro Separator: 500 µL for up to 1×10^8 cells.
    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
12. Proceed to magnetic separation (2.3).

2.3 Magnetic separation: Depletion of non-plasma cells

Depletion with LD Column

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details refer to the LD Column data sheet.
2. Prepare column by rinsing with 2 mL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 2×1 mL of buffer. Collect total effluent; this fraction contains the unlabeled pre-enriched $CD38^+$ plasma cells. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.
5. Proceed to 2.4 for the isolation of $CD38^+$ plasma cells.

Depletion with the autoMACS® Pro Separator

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

▲ Buffers used for operating the autoMACS Pro 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.

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 the following program:
   Depletion: **Depl025**
   Collect negative fraction in row B of the tube rack.
4. Proceed to 2.4 for the isolation of $CD38^+$ plasma cells.

2.4 Magnetic labeling of pre-enriched plasma cells

▲ 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 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).

▲ 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. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 50 µL of buffer.
3. Add 50 µL of CD38 MicroBeads.
4. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
5. (Optional) Add staining antibodies, e.g., 10 µL of CD38-FITC (# 130-092-259), CD19-APC (# 130-091-248), or CD138-PE (# 130-081-301), and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
6. Wash cells by adding 5–10 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Resuspend up to 10^8 cells in 500 µL of buffer.
   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
8. Proceed to magnetic separation (2.5).

2.5 Magnetic separation: Positive selection of $CD38^+$ plasma cells

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 the MS Column data sheet.
2. Prepare column by rinsing with 500 µL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 3×500 µL of buffer. Collect total effluent; this is the unlabeled cell fraction. Perform washing steps by adding buffer three times. Only add new buffer 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 purity of $CD38^+$ cells, the eluted fraction should be enriched over a second MS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

Positive selection with the autoMACS® Pro Separator

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

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

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.

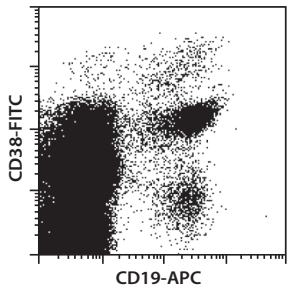
3. For a standard separation choose the following program:

   Positive selection: **Posseld2**
   Collect positive fraction in row C of the tube rack. This is the enriched CD38$^+$ plasma cell fraction.

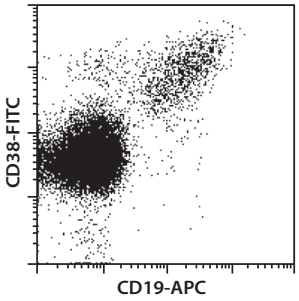
3. Example of a separation using the Plasma Cell Isolation Kit II

CD38$^+$ plasma cells were isolated from human bone marrow using the Plasma Cell Isolation Kit II, an LD Column and a MidiMACS™ Separator, and two MS Columns and an MiniMACS™ Separator. Aliquots of the different cell fractions were fluorescently stained with CD19-APC and CD38-FITC for identification of plasma cells. Cell debris and dead cells are excluded from the analysis based on scatter signals and propidium iodide fluorescence.

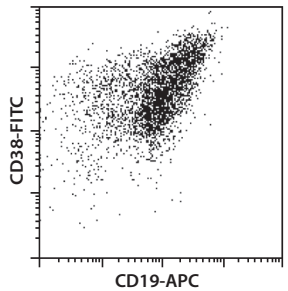
Bone marrow before separation



Pre-enriched plasma cells after depletion of non-plasma cells



Enriched CD38$^+$ plasma cells



4. References

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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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