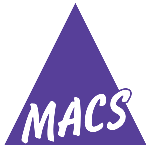

CD138+ Plasma Cell Isolation Kit
mouse

Magnetic cell sorting

Order no. 130-092-530

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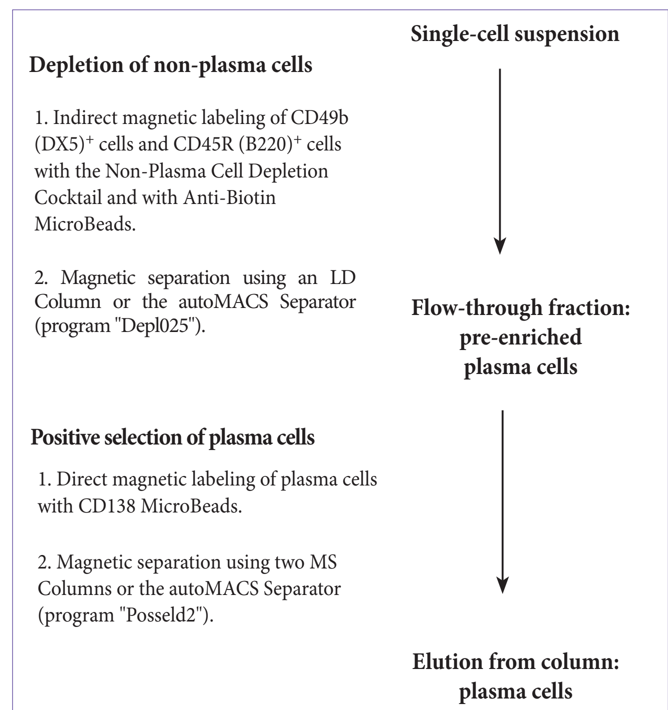
1. Description

Components	**2 mL Non-Plasma Cell Depletion Cocktail, mouse:** Cocktail of biotin-conjugated monoclonal anti-mouse antibodies against: CD49b (DX5; isotype: rat IgM), CD45R (B220; isotype: rat IgG2a). **2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibody (isotype: mouse IgG1). **2 mL CD138 MicroBeads, mouse:** MicroBeads conjugated to monoclonal anti-mouse CD138 (isotype: rat IgG2a) antibody.
Size	For 2×10^9 total cells
Product format	The Non-Plasma Cell Depletion Cocktail is supplied in a solution containing stabilizer and 0.05% sodium azide. The Anti-Biotin MicroBeads and CD138 MicroBeads are supplied as a suspension containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 4–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of MACS® separation

The isolation of mouse plasma cells is performed in a two-step procedure. First, non-plasma cells are indirectly magnetically labeled with a cocktail of biotin-conjugated antibodies and Anti-Biotin MicroBeads. The labeled cells are subsequently depleted by separation over a MACS® Column.
In the second step, plasma cells are directly labeled with CD138 MicroBeads and isolated by positive selection from the pre-enriched cell fraction.
The magnetically labeled plasma cells are retained on the column and eluted after removal of the column from the magnetic field. To achieve highest purities, the positively selected cell fraction, containing the plasma cells, is separated over a second column.



1.2 Background and product applications

The CD138+ Plasma Cell Isolation Kit, mouse was developed for the isolation of CD138+ CD45R (B220)$^{low/-}$ CD19$^{low/-}$ antibody-secreting plasma cells[1]. CD138 (Syndecan-1) is a heparan sulfate-rich integral membrane proteoglycan, which functions as a matrix receptor for interstitial collagens, fibronectin, and thrombospondin. CD138 is predominantly expressed on the surface of epithelial cells in mature mouse tissues.[2] Its expression on cells of the B cell lineage correlates with their developmental stage, location, and adhesion. In mice, CD138 is expressed on pre-B and immature B lymphocytes in the bone marrow. It is lost when B cells emigrate into the periphery, is absent on circulating and peripheral B cells, and is re-expressed

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upon B cell differentiation into plasmablasts and plasma cells.[3] The kit is suitable to isolate highly pure $CD138^+$ plasma cells from spleen, lymph nodes, and bone marrow.

Examples of applications

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

1.3 Reagent and instrument requirements

- Buffer (degassed): Prepare a solution containing PBS (phosphate buffered saline) pH 7.2, 0.5% BSA (bovine serum albumin) 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 (4 °C).

  ▲ **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 gelatine, murine serum, or fetal calf 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 plasma cells is performed on two MS Columns. Depletion and positive selection can also be performed by using the autoMACS™ Separator.

Column	max. number of labeled leukocytes	max. number of total leukocytes	Separator
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS

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

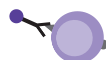
- (Optional) Fluorochrome-conjugated antibodies, e.g. CD19-FITC (# 130-092-042), CD19-PE (# 130-092-041), or CD19-APC (# 130-092-039) and CD45R (B220)-FITC (# 130-091-829), CD45R (B220)-PE (# 130-091-828), or CD45R (B220)-APC (# 130-091-843).
- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.
- (Optional) Inside Stain Kit (# 130-090-477)

2. Protocol

2.1 Sample preparation

Prepare a single-cell suspension from lymphoid tissue using standard methods.

▲ **Note:** The sample preparation should be carried out without collagenase digestion, as the digestion step could cause a weaker antibody labeling of the cells.



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.

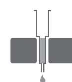
▲ Volumes for magnetic labeling given below are for up to 10^8 leukocytes. When working with fewer than 10^8 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^8 leukocytes use twice the volume of 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 # 130-041-407) to remove cell clumps which may clog the column.

1. Determine the number of leukocytes.
2. Centrifuge cells at 300×g for 10 minutes. Pipette off supernatant completely.
3. Resuspend cell pellet in 400 µL of buffer per 10^8 cells.
4. Add 100 µL of **Non-Plasma Cell Depletion Cocktail** per 10^8 cells.
5. Mix well and incubate for **10 minutes at 4–8 °C**.

   ▲ **Note:** Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
6. Wash cells by adding 5–10× labeling volume of buffer and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
7. Resuspend cell pellet in 900 µL of buffer per 10^8 cells.
8. Add 100 µL of **Anti-Biotin MicroBeads** per 10^8 cells**.**
9. Mix well and incubate for **15 minutes at 4 °C**.

   ▲ **Note:** Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
10. Wash cells by adding 5–10× labeling volume of buffer and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
11. Resuspend cell pellet in buffer:
    Depletion with LD Column: 500 µL for up to 1.25×10^8 cells
    Depletion with autoMACS: 500 µL for up to 1×10^8 cells
    ▲ **Note:** For larger 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 (see "Column data sheets").
2. Prepare column by rinsing with 2 mL of buffer.
3. Apply cell suspension onto the column.

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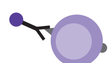
4. Collect unlabeled cells which pass through and wash column with 2 × 1 mL of buffer. Perform washing steps by adding buffer successively once the column reservoir is empty. Collect total effluent. This contains the unlabeled pre-enriched plasma cell fraction.
5. Proceed to 2.4 for the isolation of plasma cells.

Depletion with the autoMACS™ Separator

▲ Refer to the "autoMACS User Manual" for instructions on how to use the autoMACS™ Separator.

1. Prepare and prime the autoMACS Separator.
2. Place the tube containing the magnetically labeled cells in the autoMACS Separator. Choose separation program "Depl025".
3. Collect the unlabeled fraction (outlet port "neg1"). This is the pre-enriched plasma cell fraction.
4. Proceed to 2.4 for the enrichment of plasma cells.



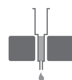
2.4 Magnetic labeling of plasma cells

▲ Volumes for magnetic labeling given below are for an initial starting cell number of up to 10^8 leukocytes. For larger initial cell numbers, scale up volumes accordingly.

1. Centrifuge the cells at 300×g for 10 minutes. Pipette off supernatant completely.
2. Resuspend cell pellet in 400 µL of buffer per 10^8 cells.
3. Add 100 µL of **CD138 MicroBeads** per 10^8 cells.
4. Mix well and incubate for **15 minutes at 4–8°C.**

   ▲ **Note:** Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
5. (Optional) Add staining antibodies at the titer recommended by the manufacturer and incubate for 5 minutes at 4–8°C.
6. Wash cells by adding 5–10× labeling volume of buffer and centrifuge at 300×g for 10 minutes. Pipette off 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 plasma cells

Positive selection with MS Columns

1. Place MS Column in the magnetic field of a suitable MACS Separator (see "Column data sheets").
2. Prepare column by rinsing with 500 µL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells which pass through and wash column with 3 × 500 µL of buffer. Perform washing steps by adding buffer three times once 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 fraction with magnetically labeled cells (plasma cells) by firmly applying the plunger supplied with the column.

7 Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

Positive selection with the autoMACS Separator

▲ Refer to the "autoMACS User Manual" for instructions on how to use the autoMACS Separator.

1. Prepare and prime the autoMACS Separator.
2. Place the tube containing the magnetically labeled cells in the autoMACS Separator. Choose separation program "Posseld2".
3. Collect the positive fraction (outlet port "pos2"). This is the enriched plasma cell fraction.

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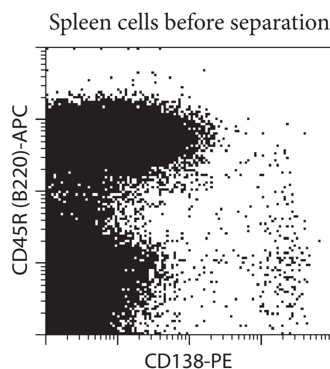
3. Examples of plasma cell isolations

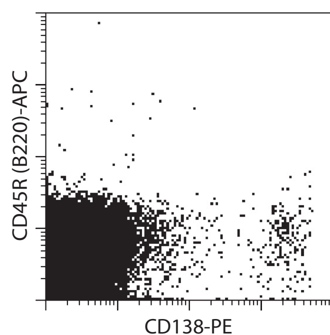
3.1 Separation of plasma cells using the CD138+ Plasma Cell Isolation Kit

Plasma cells were isolated from a mouse spleen cell suspension by using the CD138+ Plasma Cell Isolation Kit, an LD and two MS Columns, a MidiMACS™ and a MiniMACS™ Separator. The cells were fluorescently stained with CD45R (B220)-APC, CD19-FITC, and CD138-PE. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

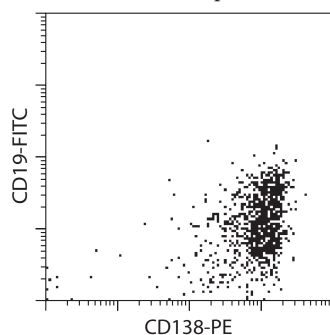
Spleen cells before separation



Pre-enriched plasma cells after depletion of CD49b (DX5)+ cells and CD45R (B220)+ cells

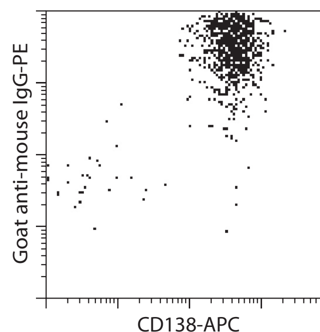


Isolated CD138+ plasma cells



3.2 Intracellular staining of isolated CD138+ plasma cells

Plasma cells were isolated from a mouse bone marrow cell suspension by using the CD138+ Plasma Cell Isolation Kit. The CD138+ plasma cells were stained intracellularly with goat anti-mouse IgG-PE by applying the solid-phase intracellular staining procedure (Inside Stain Kit # 130-090-477). Please visit our website at www.miltenyibiotec.com for a detailed protocol of the solid-phase intracellular staining technology. Additionally, the cell surface of the CD138+ plasma cells was fluorescently stained with CD138-APC. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.



4. References

1. Wehrli, N. *et al.* (2001) Changing responsiveness to chemokines allows medullary plasmablasts to leave lymph nodes. Eur. J. Immunol. 13: 609-616.
2. Kim, C.W. *et al.* (1994) Members of the syndecan family of heparan sulfate proteoglycans are expressed in distinct cell-, tissue-, and development-specific patterns. Mol. Biol. Cell 5: 797-805.
3. Sanderson, R.D. *et al.* (1989) B lymphocytes express and lose syndecan at specific stages of differenciation. Cell Regulation 1: 27-35.

Warning

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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