For further information refer to our website
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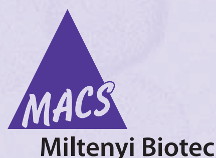
140-000-102.05



CD138 MicroBeads
human

For 2×10⁹ total cells,
up to 100 separations

Order no. 130-051-301

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140-000-102.05

1. Description

Components	**2 mL CD138 MicroBeads, human:** MicroBeads conjugated to monoclonal anti-human CD138 antibodies (isotype: mouse IgG1).
Capacity	For 2×10⁹ total cells, up to 100 separations.
Product format	CD138 MicroBeads 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 dates are indicated on the vial labels.

1.1 Principle of the MACS® Separation

First, the CD138⁺ cells are magnetically labeled with CD138 MicroBeads. Then, the cell suspension is loaded onto a MACS® Column which is placed in the magnetic field of a MACS Separator. The magnetically labeled CD138⁺ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD138⁺ cells. After removing the column from the magnetic field, the magnetically retained CD138⁺ cells can be eluted as the positively selected cell fraction.
If a very high purity is desired, we recommend the depletion of CD20⁺ B cells with CD20 MicroBeads prior to positive selection with CD138 MicroBeads. For details see the special protocol "Isolation of CD138⁺ plasma cells" at www.miltenyibiotec.com/protocols.

140-000-102.05

1.2 Background information

CD138 MicroBeads have been developed for the isolation of plasma cells from human peripheral blood mononuclear cells (PBMCs), bone marrow, or lymphoid tissue.

CD138, also known as syndecan-1, is expressed on normal and malignant human plasma cells and commonly used as a universal marker for their identification.[1] CD138 is also expressed on basolateral surfaces of endothelial cells, embryonic mesenchymal cells, vascular smooth muscle cells, and neural cells.[2,3]

In mouse, CD138 is expressed on plasma cells but also B cell precursors in the bone marrow.[4]

1.3 Applications

- Isolation or depletion of plasma cells from PBMCs, bone marrow, leukaphereses harvests, or single-cell suspensions from lymphoid tissue, for phenotypical and functional characterization, for example, by flow cytometric analysis, molecular biology studies such as PCR, microarrays, or protein analysis.
- Positive selection or depletion of malignant plasma cells from bone marrow of patients with multiple myeloma.
- Positive selection and subsequent solid phase intracellular staining of normal and malignant plasma cells with the Inside Stain Kit, for example, for kappa or lambda light chains of immunoglobulins.

1. Description

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.
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD138-PE (# 130-081-301), CD138-APC (# 130-091-250), CD19-PE (# 130-091-247), CD19-APC (# 130-091-248), CD56-APC (# 130-090-843), or CD45-FITC (# 130-080-202). For more information about other fluorochrome conjugates see www.miltenyibiotec.com.
- (Optional) Antibodies specific for intracellular antigens, e.g., Anti-Ig κ Light Chain-FITC (# 130-093-053) or Anti-Ig λ Light Chain-APC (# 130-093-038). For more information about other fluorochrome conjugates see www.miltenyibiotec.com.
- (Optional) Inside Stain Kit (# 130-090-477) for intracellular staining of CD138$^+$ cells.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells without fixation.

- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.
- MACS Columns and MACS Separators: CD138$^+$ cells can be enriched by using MS, LS, or XS Columns or depleted with the use of LD, CS, or D Columns. Cells which strongly express the CD138 antigen can also be depleted using MS, LS, or XS Columns. Positive selection or depletion can also be performed by using the autoMACS or the autoMACS Pro Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
XS	10^9	2×10^{10}	SuperMACS
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
CS	2×10^8		VarioMACS, SuperMACS
D	10^9		SuperMACS
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS, autoMACS Pro

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

1. Description

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™. For details see the General Protocols section of the respective separator user manual. The General Protocols are also available at www.miltenyibiotec.com/protocols.

▲ **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 see the General Protocols section of the respective separator user manual. The General Protocols are also available 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

▲ 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 2×10^7 total cells. When working with fewer than 2×10^7 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 4×10^7 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, # 130-041-407) to remove cell clumps which may clog the column. Wet 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.

▲ It has been reported that normal plasma cells are $CD19^+$ and $CD56^-$, whereas myeloma cells are often $CD19^-$ and $CD56^+$.[6] For differentiation between normal and malignant cells, cells can be stained with CD19- and CD56-specific antibodies.

▲ After fixation and permeabilization, $CD138^+$ cells can also be counterstained for intracellular antigens, e.g., immunoglobulin light chains (see 5. Appendix).[7]

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 80 µL of buffer per 2×10^7 total cells.
4. Add 20 µL of CD138 MicroBeads per 2×10^7 total cells.
5. Mix well and incubate for 15 minutes in the refrigerator (2−8 °C).
6. (Optional) Add staining antibodies, e.g., 10 µL of CD138-PE (# 130-081-301), and incubate for 5 minutes in the dark in the refrigerator (2−8 °C).
7. Wash cells by adding 1–2 mL of buffer per 2×10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend up to 10^8 cells in 500 µL of buffer.
   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
   ▲ **Note:** For depletion with LD Columns, resuspend up to 1.25×10^8 cells in 500 µL of buffer.
9. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of $CD138^+$ cells. For details see table in section 1.4.

▲ The binding capacities of the columns mentioned in the separation protocols represent guidelines. Due to the large size of myeloma cells in individual cases the maximal binding capacity of the columns may be decreased (e.g. 3-fold decreased for cells of the cell line U266).

Magnetic separation with MS or 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 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. 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. Remove column from the separator and place it on a suitable collection tube.
6. Pipette the appropriate amount of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
   MS: 1 mL LS: 5 mL
7. (Optional) To increase the purity of $CD138^+$ cells, the eluted fraction can be enriched over a second MS or LS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

Magnetic separation with XS Columns

For instructions on the column assembly and the separation refer to the XS Column data sheet.

Depletion with LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details see 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 is the unlabeled $CD138^-$ cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.

Depletion with CS Columns

1. Assemble CS Column and place it in the magnetic field of a suitable MACS Separator. For details see CS Column data sheet.
2. Prepare column by filling and rinsing with 60 mL of buffer. Attach a 22G flow resistor to the 3-way stopcock of the assembled column. For details see CS Column data sheet.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 30 mL buffer from the top. Collect total effluent; this is the unlabeled $CD138^-$ cell fraction.

Depletion with D Columns

For instructions on column assembly and separation refer to the D Column data sheet.

Magnetic separation with the autoMACS™ Separator or the autoMACS™ Pro Separator

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

▲ Buffers used for operating the autoMACS Separator or 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.

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 (“Possel”) or port pos2 (“Posseld”).
3. For a standard separation choose one of the following programs:

   Positive selection: “Possel” or “Posseld”
   Collect positive fraction from outlet port pos1 (“Possel”) or port pos2 (“Posseld”).

   Depletion: “Depletes”
   Collect negative fraction from outlet port neg1.

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:

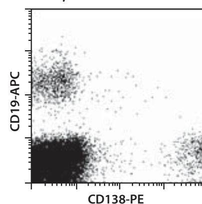
   Positive selection: “Possel” or “Posseld”
   Collect positive fraction in row C of the tube rack.

   Depletion: “Depletes”
   Collect negative fraction in row B of the tube rack.

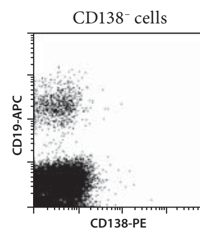
3. Examples of separations using the CD138 MicroBeads

A cell mixture consisting of human PBMCs from a healthy donor and cells of a myeloma cell line (U266) was separated into $CD138^+$ and $CD138^-$ cells using the CD138 MicroBeads, two MS Columns, and a MiniMACS™ Separator. Cells are fluorescently stained with CD138-PE (# 130-081-301) and CD19-APC (# 130-091-248). Cell debris and dead cells are excluded from the analysis based on scatter signals and PI fluorescence.

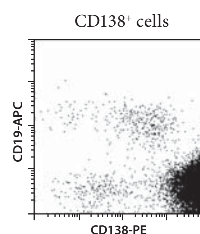
PBMCs and myeloma cells before separation



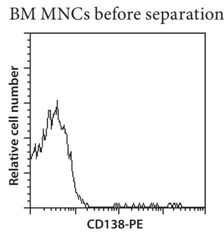
$CD138^-$ cells

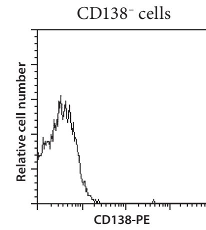


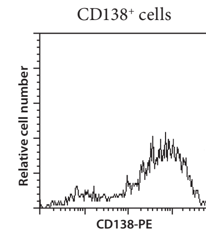
$CD138^+$ cells



Bone marrow mononuclear cells (BM MNCs) from a patient with multiple myeloma were separated using the CD138 MicroBeads, an MS Column, and a MiniMACS Separator. Cells are fluorescently stained with CD138-PE (# 130-081-301). Cell debris and dead cells are excluded from the analysis based on scatter signals and PI fluorescence.







4. References

1. Bataille, R. *et al.* (2006) The phenotype of normal, reactive and malignant plasma cells. Identification of "many and multiple myelomas" and of new targets for myeloma therapy. Haematologica 91: 1234–1240.
2. Bernfield, M. *et al.* (1992) Biology of the syndecans: a family of transmembrane heparan sulfate proteoglycans. Annu. Rev. Cell Biol. 8: 365–393.
3. Couchman, J. R. and Woods, A. (1996) Syndecans, signaling, and cell adhesion. J. Cell. Biochem. 61: 578–584.
4. Sanderson, R. D. *et al.* (1989) B lymphocytes express and lose syndecan at specific stages of differentiation. Cell regulation 1: 27–35.

5. Appendix

Isolated CD138$^+$ cells can be subsequently analyzed phenotypically by using the Inside Stain Kit and by staining them while immobilized on a MACS Column (5.1) in suspension (5.2) or on slides (5.3).

5.1 Solid phase intracellular staining

5.1.1 Protocol overview

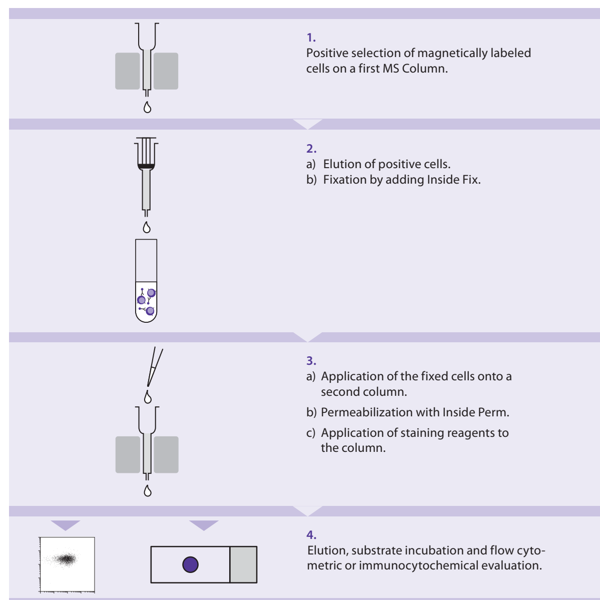


Figure: In-column intracellular staining of cells isolated by MACS® Technology.

5.1.2 Reagent and instrument requirements

- Inside Stain Kit (# 130-090-477).
- 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.
- Antibodies specific for intracellular antigens, e.g., Anti-Ig κ Light Chain-FITC (# 130-093-053) or Anti-Ig λ Light Chain-APC (# 130-093-038). For more information about other fluorochrome conjugates see www.miltenyibiotec.com.

5.1.3 Protocol

▲ Staining of the cells according to surface antigens (e.g. CD138-PE, # 130-081-301) can be performed either in parallel with intracellular staining (protocol step 7.), or before or after intracellular staining in buffer.

1. After separation, centrifuge cells at 300×g for 10 minutes. Aspirate supernatant completely and resuspend up to 10^7 cells in 500 µL of buffer.
2. Add 500 µL of Inside Fix to the magnetically labeled cell fraction and incubate for 20 minutes at room temperature. The final fixation volume is 1 mL.
3. Place an MS Column in the magnetic field of a suitable MACS Separator and prepare column by rinsing with 500 µL of buffer.
4. Apply the fixed cell suspension onto the column, let cell suspension completely enter the column matrix, and immediately wash with 500 µL of buffer.
5. Permeabilize cells by washing the MS Column with 500 µL of Inside Perm.
6. Dilute fluorochrome-conjugated primary antibody according to the manufacturer's recommendations in Inside Perm, e.g. dilute 10 µL of Anti-Ig κ Light Chain-FITC (# 130-093-053) in 100 µL of Inside Perm.
7. Apply 110 µL of antibody solution onto column and incubate for 10 minutes at room temperature.

   ▲ **Note:** The MACS Column has a flow-stop mechanism that will retain the solution in the column.

8. (Optional for indirect stainings) Rinse column with 2×500 µL of Inside Perm, apply 100 µL of the secondary antibody diluted at appropriate titer in Inside Perm and incubate for 10 minutes at room temperature. For application of additional staining reagents repeat steps 6–8.
9. Wash cells by rinsing the column with 500 µL of Inside Perm followed by 500 µL buffer.
10. Remove MS Column from the separator and place it on a suitable collection tube.
11. Pipette 500 µL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
12. Cells are now ready for analysis. Store cells at 2–8 °C in the dark until analysis. Mix well before flow cytometric data acquisition.

    ▲ **Note:** Samples may be stored at 2–8 °C in the dark for up to 24 hours.

    ▲ **Note:** Do not use propidium iodide (PI) or 7-AAD staining.

5.2 Intracellular staining of cells in suspension

5.2.1 Reagent and instrument requirements

- Inside Stain Kit (# 130-090-477).
- 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.
- Antibodies specific for intracellular antigens, e.g., Anti-Ig κ Light Chain-FITC (# 130-093-053) or Anti-Ig λ Light Chain-APC (# 130-093-038). For more information about other fluorochrome conjugates see www.miltenyibiotec.com.

5.2.2 Protocol

▲ It is recommended to stain 10^6 cells per sample. When working with up to 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 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. After separation, centrifuge cells at 300×g for 10 minutes. Aspirate supernatant completely and resuspend up to 10^7 cells in 500 µL of buffer.
2. Add 500 µL of Inside Fix (Inside Stain Kit). Mix well and incubate for 20 minutes at room temperature.
3. Centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
4. Wash cells by adding 1 mL of buffer and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
   ▲ **Note:** Fixed cells may be stored at 2–8 °C for up to 1 week.
5. Dilute fluorochrome-conjugated antibody according to the manufacturer's recommendations in Inside Perm, e.g. dilute 10 µL of Anti-Ig κ Light Chain-FITC (# 130-093-053) in 100 µL of Inside Perm.
6. Resuspend cell pellet in diluted staining antibody.
7. Mix well and incubate for 10 minutes in the dark at room temperature.
8. Wash cells by adding 1 mL of Inside Perm and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
9. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy. Store cells at 2–8 °C in the dark until analysis. Mix well before flow cytometric data acquisition.
   ▲ **Note:** Samples may be stored at 2–8 °C in the dark for up to 24 hours.
   ▲ **Note:** Do not use propidium iodide (PI) or 7-AAD staining.

5.3 Intracellular staining of cells on slides

5.3.1 Reagent and instrument requirements

- Inside Stain Kit (# 130-090-477).
- Phosphate buffered saline, pH 7.2.
- Antibodies specific for intracellular antigens, e.g., Anti-Ig κ Light Chain-FITC (# 130-093-053) or Anti-Ig λ Light Chain-PE (# 130-093-038). For more information about other fluorochrome conjugates see www.miltenyibiotec.com.
- 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.
- Mounting medium.
- Cytocentrifuge.
- Slides and coverslips.
- Staining trough, type Hellendahl.
- Humidity chamber.

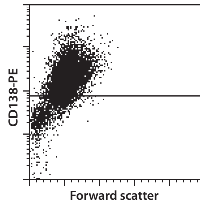
5.3.2 Protocol

1. Resuspend an appropriate number of cells in buffer at a final concentration of 10^5–10^6 cells/mL.
2. Transfer an aliquot of 50–100 µL onto a slide using a cytocentrifuge.
3. Let the slides dry for 20 minutes at 20–25 °C.
4. Fix cells in Inside Fix for 20 minutes at 20–25 °C using a staining trough.
5. Wash slides in buffer using a staining trough.
   ▲ **Note:** Slides can be stored in buffer supplemented with 0.5% BSA and 0.05% NaN_3 at 4 °C in the dark until staining.
6. Incubate slides in Inside Perm for 10 minutes at 20–25 °C using a staining trough.
7. Wipe slides dry but exclude cell area.
8. Dilute fluorochrome-conjugated antibody according to the manufacturer's recommendations in Inside Perm, e.g. dilute 10 µL of Anti-Ig κ Light Chain-FITC (# 130-093-053) in 100 µL of Inside Perm.
9. Add 250 µL of diluted antibody solution to the cell area and incubate for 10 minutes in the dark at 20–25 °C using a humidity chamber.
10. Wash slides in PBS using a staining trough.
11. Let slides dry. Then cover cells with mounting medium and coverslid.
12. Proceed to fluorescence analysis.

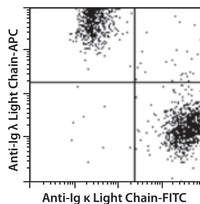
5.4 Example of solid phase intracellular staining of isolated CD138+ plasma cells using the Inside Stain Kit

CD138+ plasma cells isolated from PBMCs of a normal donor (A and B) or from human bone marrow (C and D) were stained with Anti-Ig κ Light Chain-FITC (# 130-093-053), CD138-PE, and Anti-Ig λ Light Chain-APC (# 130-093-043) using the Inside Stain Kit and the Solid phase intracellular staining protocol (5.1).

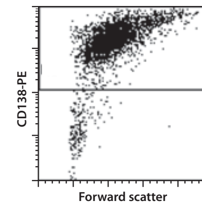
A) Gating on CD138+ cells



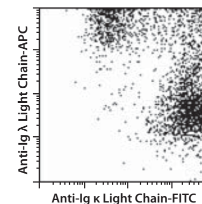
B) Staining of gated CD138+ cells with Anti-Ig λ Light Chain-APC versus Anti-Ig κ Light Chain-FITC.



C) Gating on CD138+ cells



D) Staining of gated CD138+ cells with Anti-Ig λ Light Chain-APC versus Anti-Ig κ Light Chain-FITC.



All protocols and data sheets are available at **www.miltenyibiotec.com.**

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.

Warranty

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