CD138 antibodies
human

Contents

1. Description

Components	1 mL monoclonal CD138 antibodies, human conjugated to various dyes.
	PE 130-081-301 APC 130-091-250 VioGreen™ 130-096-911
Clone	B-B4 (isotype: mouse IgG1).
Capacity	100 tests or up to 10^9 total cells.
Product format	Antibodies 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 Background information

The CD138 antigen is a transmembrane heparan sulfate proteoglycan, also known as Syndecan-1. It interacts with extracellular matrix proteins, cell surface molecules, and soluble proteins. CD138 is expressed on normal and malignant plasma cells, but not on virgin/naive B cells, memory B cells, T cells, or monocytes. Further, CD138 is expressed on the basolateral surfaces of epithelial cells, embryonic mesenchymal cells, vascular smooth muscle cells, endothelial cells, and neural cells. [1-5]

1.2 Applications

- Identification and enumeration of normal and malignant human plasma cells in peripheral blood, bone marrow, leukapheresis harvests, and single-cell suspensions of immunoreactive tissue by flow cytometry or fluorescence microscopy.
- Immunophenotyping of malignant hematopoietic cells in peripheral blood, bone marrow, and in leukapheresis harvests. Normal and malignant cells may be distinguished by counterstaining with CD19 or CD56 antibodies.[6] After fixation and permeabilization cells can be counterstained for intracellular antigens, e.g., immunoglobulin[7], by using the Inside Stain Kit (# 130-090-477).
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Human $CD138^+$ cells can be isolated by using, for example, CD138 MicroBeads, human (# 130-051-301).[8]

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD138 conjugates is **1:11 for up to 10^7 cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry. For CD138 MicroBead-labeled cells use the same dilution. CD138-VioGreen for staining of cells labeled with CD138 MicroBeads is not recommended.

1.4 Reagent 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).

  ▲ **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.
- (Optional) CD19-FITC (# 130-091-328) or CD19-PE (# 130-091-247). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Mouse IgG1 isotype control antibodies conjugated to, e.g., PE (# 130-092-212). For more information about isotype control antibodies refer to www. miltenyibiotec.com.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells without fixation.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

2. General protocol for immunofluorescent staining

▲ CD138-VioGreen staining is only recommended for cells which process a bright CD138 expression, e.g., malignant plasma cells or myeloma cell lines (U266, MOLP-8).

▲ Volumes given below are for **up to 10^7** nucleated 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 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.

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3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.

4. Add 10 µL of the CD138 antibody.

5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
   ▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.

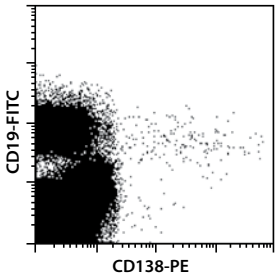
6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

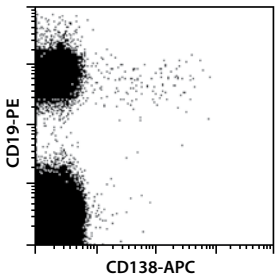
3. Examples of immunofluorescent staining with CD138 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD138 antibodies conjugated to PE (A) or APC (B) as well as with CD19-FITC (# 130-091-328) or CD19-PE (# 130-091-247) and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

A)

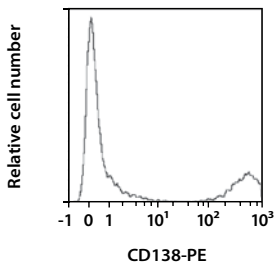


B)

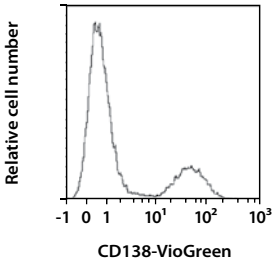


A mixture of human PBMCs and cells of the human myeloma cell line U266 were stained with CD138-PE (C) or CD138-VioGreen (D) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

C)



D)



4. References

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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.

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