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1. Description

Components	1 mL monoclonal CD11c antibodies, mouse conjugated to various dyes.	
	FITC	130-091-842
	PE	130-091-830
	APC	130-091-844
	VioBlue®	130-097-337
Clone	N418 (isotype: hamster IgG).	
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 mouse CD11c antigen is expressed on dendritic cells in lymphoid organs and blood, on Langerhans cells in epidermis, on dendritic cell progenitors in bone marrow, and on *in vitro* generated bone marrow derived dendritic cells.^{1–3} In spleen and lymph node, CD11c is expressed at high levels on conventional CD11c⁺CD45R⁻ mPDCA-1⁻ dendritic cells, and at moderate levels on CD11c⁺CD45R⁺ mPDCA-1⁺ plasmacytoid dendritic cells. CD11c is reported to be weakly expressed on NK cells, B cells, and T cell subsets.

The CD11c antibody clone N418 reacts with the integrin α_x subunit of the leukocyte integrin gp150,95 (CD11c/CD18).³ About 1–3% of splenocytes, 2% of bone marrow cells, and <1% of lymph node cells and thymocytes express CD11c.

1.2 Applications

- Identification and enumeration of CD11c⁺ cells by flow cytometry or fluorescence microscopy.

- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy, for example:
 - Positive selection or depletion of mouse CD11c⁺ dendritic cells by using CD11c MicroBeads, mouse (# 130-052-001);
 - Isolation of mouse CD4⁺CD11c⁺ dendritic cells by using the CD4⁺ Dendritic Cell Isolation Kit, mouse (# 130-091-262);
 - Isolation of mouse CD8⁺CD11c⁺ dendritic cells by using the CD8⁺ Dendritic Cell Isolation Kit, mouse (# 130-091-169);
 - Isolation of mouse CD11c⁺CD45R⁺ mPDCA-1⁺ plasmacytoid dendritic cells by using the Plasmacytoid Dendritic Cell Isolation Kit II, mouse (# 130-092-786).

1.3 Recommended antibody dilution

The recommended antibody dilution for all CDxy conjugates is **1:11 for up to 10⁷ cells/100 μ L** of buffer for labeling of cells and analysis by flow cytometry. For CD11c MicroBead-labeled cells use the same dilution.

The antibody is suited for staining of formaldehyd-fixed cells.

Cells should be stained prior to fixation, if formaldehyde is used as a fixative.

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 mouse serum albumin, mouse serum, or fetal bovine serum (FBS). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- CD11c MicroBeads, mouse (# 130-052-001) for magnetic labeling.
- (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

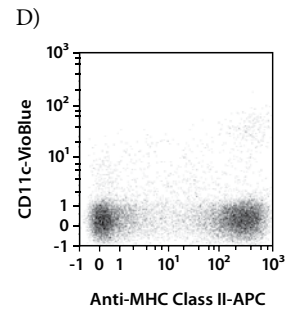
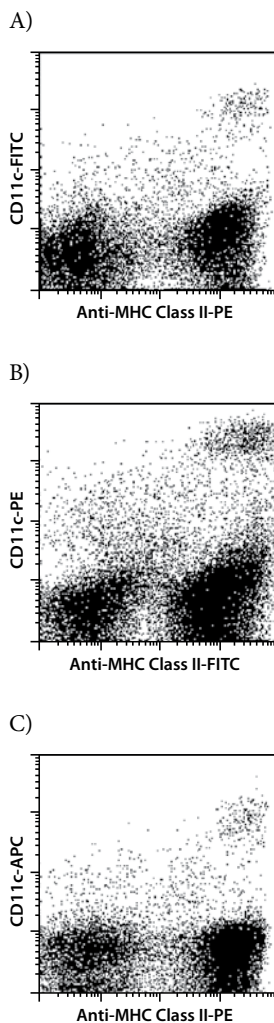
▲ Volumes given below are for up to 10⁷ nucleated cells. When working with fewer than 10⁷ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10⁷ nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.

2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10⁷ nucleated cells per 100 μL of buffer.
4. Add 10 μL of the CD11c 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 CD11c antibodies

Mouse spleen cells were stained with CD11c antibodies conjugated to FITC (A), PE (B), APC (C), or VioBlue (D) as well as with Anti-MHC Class II-PE, mouse (# 130-091-368) or Anti-MHC Class II-FITC, mouse (# 130-081-601) or Anti-MHC Class II-APC and analyzed by flow cytometry. The MACSQuant® Analyzer was used for the analysis shown in picture (D). Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



For more examples please refer to the respective product page at www.miltenyibiotec.com.

4. References

1. Metlay J.P. *et al.* (1990) The Distinct Leukocyte Integrins of Mouse Dendritic Cells as Identified With New Hamster Monoclonal Antibodies. *J. Exp. Med.* 171: 1753–1771.
2. Larson R.S. and Springer T.A. (1990) Structure and function of leukocyte integrins. *Immunol. Rev.* 114: 181–217.
3. Bilslund C.A.G. *et al.* (1994) The integrin p150, 95 (CD11c/CD18) as a receptor for iC3b. Activation by a heterologous beta subunit and localization of a ligand recognition site to the I domain. *J. Immunol.* 152: 4582–4589.

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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