

Contents

1. Description
 - 1.1 Background information
 - 1.2 Applications
 - 1.3 Recommended antibody dilution
 - 1.4 Reagent requirements
2. General protocol for immunofluorescent staining
3. Example of immunofluorescent staining with CD11c antibodies
4. References

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 Monoclonal CD11c antibodies, human conjugated to:

Conjugate	Order no. 1 mL (100 tests)	Order no. 300 µL (30 tests)
FITC	130-092-410	130-099-236
PE	130-092-411	130-099-212
APC	130-092-412	–
VioBlue®	130-097-328	130-099-716
PE-Vio770™	130-099-711	130-099-712
APC-Vio770™	130-100-238	130-100-214
PerCP-Vio700™	130-103-790	130-103-860
Biotin	130-092-413	130-099-694
pure	130-092-414	–

Clone MJ4-27G12 (isotype: mouse IgG2b).

Capacity 1 mL: 100 tests or up to 10⁹ total cells
300 µL: 30 tests or up to 3×10⁸ total cells.
The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL.

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

- Antigen: CD11c
- Synonym: 95; CR4; integrin αX; ITGAX; p150
- Expression patterns: This CD11 antibody reacts with human CD11c, a 145–150 kD type I transmembrane glycoprotein also known as integrin αX or CR4. It is expressed on monocytes, macrophages, NK cells, granulocytes, myeloid dendritic cells (MDCs), and subsets of T and B cells. On myeloid dendritic cells, CD11c is highly expressed on type 1 myeloid dendritic cells (CD1c (BDCA-1)⁺ CD123^{low} MDC1s) and low on type 2 myeloid dendritic cells (CD1c(BDCA-1)[–] CD123[–] MDC2s). This CD11 antibody reacts with human CD11c, a 145–150 kD type I transmembrane glycoprotein also known as integrin αX or CR4. It is expressed on monocytes, macrophages, NK cells, granulocytes, myeloid dendritic cells (MDCs), and subsets of T and B cells. On myeloid dendritic cells, CD11c is highly expressed on type 1 myeloid dendritic cells (CD1c (BDCA-1)⁺ CD123^{low} MDC1s) and low on type 2 myeloid dendritic cells (CD1c(BDCA-1)[–] CD123[–] MDC2s). CD11c, also known as integrin integrin αX or CR4, has been reported to play a role in adhesion and CTL killing through its interactions with fibrinogen, CD54, and iC3b.

1.2 Applications

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

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD11c conjugates is **1:11 for up to 10⁷ cells/100 µL** of buffer for labeling of cells and subsequent analysis by flow cytometry.

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). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.

- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Tandem Signal Enhancer, human (# 130-099-888) to reduce non-specific binding of tandem dye-conjugated antibodies to human cells, especially to monocytes.
- (Optional) Conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with CD11c-Biotin.
- (Optional) For antibodies for additional staining or for isotype control, refer to www.miltenyibiotec.com/antibodies.

- (Optional) Propidium Iodide Solution (# 130-093-233) 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.

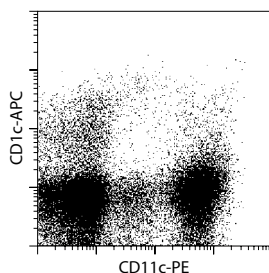
2. General protocol for immunofluorescent staining

Volumes given below are for **up to 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 $300 \times g$ for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 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 ($2-8^\circ\text{C}$).
 ▲ **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 $300 \times g$ for 10 minutes. Aspirate supernatant completely.
7. (Optional) If CD11c-Biotin was used, resuspend the cell pellet in 100 μL of buffer, add 10 μL of anti-biotin antibody, and continue as described in steps 5 and 6.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Example of immunofluorescent staining with CD11c antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD11c antibodies conjugated to PE as well as with CD1c (BDCA-1)-APC and analyzed by flow cytometry. 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/antibodies.

4. References

1. Barclay, A. N. *et al.* (1997) In: The Leukocyte Antigen Facts Book, Academic Press, San Diego, CA. (2nd edition): 161–162.
2. Dzionek, A. *et al.* (2000) BDCA-2, BDCA-3, BDCA-4: Three markers for distinct subsets of dendritic cells in human peripheral blood. *J. Immunol.* 165: 6037–6046.
3. Bendiss-Vermare, N. *et al.* (2001) Human thymus contains IFN-alpha-producing CD11c(-), myeloid CD11c(+), and mature interdigitating dendritic cells. *J. Clin. Invest.* 107: 835–844.

Refer to www.miltenyibiotec.com for all data sheets and protocols.

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