

CD1c (BDCA-1) antibodies human

CD1c (BDCA-1)-FITC	130-090-507
CD1c (BDCA-1)-PE	130-090-508
CD1c (BDCA-1)-APC	130-090-903
CD1c (BDCA-1)-Biotin	130-090-692
CD1c (BDCA-1) pure	130-090-695

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1. Description

Clone AD5-8E7 (isotype: mouse IgG2a).¹

Product format 1 mL CD1c (BDCA-1) antibodies, human: monoclonal CD1c (BDCA-1) antibodies conjugated to fluorescein-isothiocyanate (FITC), R-phycoerythrin (PE), allophycocyanin (APC), or biotin (Biotin). The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL. Antibodies are supplied in a solution containing stabilizer and 0.05% sodium azide.

Product size 100 tests (for up to 10⁹ nucleated cells).

Storage Store protected from light at 4–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background and product applications

The CD1c (BDCA-1) antigen¹ is expressed on a major subpopulation of human myeloid blood dendritic cells (about 0.3% of blood leukocytes). In blood, CD1c (BDCA-1)⁺ myeloid dendritic cells are CD11c^{high}, CD123^{low}, CD4⁺, Lin⁻, CD45RO⁺, CD2⁺, CD16⁻, CD141 (BDCA-3)^{low}, CD303 (BDCA-2)⁻, and CD304 (BDCA-4/Neuropilin-1)⁻.¹⁻⁴ They express myeloid markers (CD13, CD33) as well as Fc receptors (CD32, CD64, FcεRI) and are of monocytoid appearance.¹ A minor proportion of CD1c (BDCA-1)⁺ myeloid dendritic cells expresses CD14 and CD11b. In blood, CD1c (BDCA-1) is also expressed on a subpopulation of CD19⁺ small resting B lymphocytes. CD1c (BDCA-1) expression has also been detected on cortical thymocytes, on Langerhans cells⁷, and on CD1a⁺ dendritic cells generated *ex vivo* from monocytes or hematopoietic precursor cells. CD1c (BDCA-1)⁺ myeloid dendritic cells have been designated as type 1 myeloid dendritic cells (MDC1s).

Examples of applications

- Identification and enumeration of CD1c (BDCA-1)⁺ myeloid dendritic cells in peripheral blood or lymphoid and non-lymphoid tissue by immunofluorescent or immunocytochemical staining and flow cytometric or microscopic analysis. The antibodies are suitable to stain of fresh or formaldehyde-fixed cells in suspension and to stain, for example, air-dried, acetone-fixed cryosections. They are not suitable for paraffin-embedded tissue sections.

- CD1c (BDCA-1) antibodies were used, for example, to quantify and characterize CD1c (BDCA-1)⁺ myeloid dendritic cells in blood¹⁻⁵ and to localize of CD1c (BDCA-1)⁺ myeloid dendritic cells in lymphoid and non-lymphoid tissue^{6,7}.

1.2 Examples of staining concentrations for human cells.

CD1c (BDCA-1) conjugate	FITC	PE	APC	Biotin
	Recommended antibody dilution			
Flow cytometry^a				
- in general	1:11	1:11	1:11	1:11
- formaldehyde-fixed cells ^b	1:11	1:11	1:11	1:11
- in combination with the CD1c (BDCA-1) ⁺ Dendritic Cell Isolation Kit (# 130-090-506) ^c	n. r.	1:11	1:11	n. r.
Immunohistochemistry^d				
a) Given antibody dilutions are for a cell concentration of up to 1 × 10 ⁸ cells/mL buffer. b) For optimal results, cells have to be stained prior to fixation. c) Cells have to be stained after magnetic separation. d) For immunohistochemical staining, the optimal antibody dilution has to be tested. n. r. not recommended				

- The CD1c (BDCA-1) antibody is reported to react with rhesus monkey (*Macaca mulatta*) and cynomolgus monkey (*Macaca fascicularis*) cells.⁸

1.3 Reagent requirements

- Buffer: Prepare a solution containing PBS (phosphate buffered saline) pH 7.2, 0.5% BSA (bovine serum albumin) and 2 mM EDTA, e.g. by diluting MACS® BSA Stock Solution (# 130-091-376) 1:20 with autoMACS™ Rinsing Solution (# 130-091-222). Keep buffer cold (4–8 °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 calf serum. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- FcR Blocking Reagent (# 130-059-901) to avoid Fc receptor-mediated cell staining.
- (Optional) Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with CD1c (BDCA-1)-Biotin.
- (Optional) PI (propidium iodide) or 7-AAD for flow cytometric exclusion of dead cells without cell fixation. For cell fixation and flow cytometric exclusion of dead cells, the Fixation and Dead Cell Discrimination Kit (# 130-091-163) is recommended.

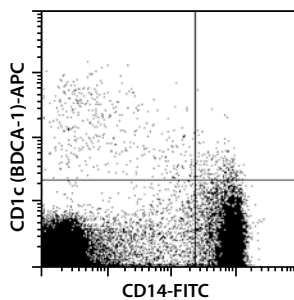
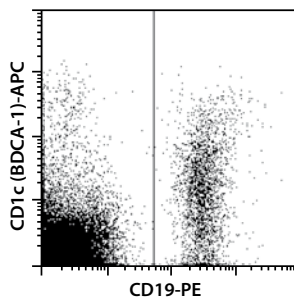
2. General protocol for immunofluorescent staining

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

1. Resuspend up to 10^7 nucleated cells per 80 μ L of buffer.
2. Add 20 μ L of FcR Blocking Reagent.
3. Add 10 μ L of CD1c (BDCA-1) antibody.
4. Mix well and incubate for 10 min in the dark at 4–8 °C.
▲ **Note:** Working on ice requires increased incubation time. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
5. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge for 10 min at $300 \times g$ and 4–8 °C. Pipette off supernatant completely.
6. (Optional) If CD1c (BDCA-1)-Biotin was used, resuspend the cell pellet in 100 μ L buffer, add 10 μ L Anti-Biotin antibody (Anti-Biotin-FITC # 130-090-857, Anti-Biotin-PE # 130-090-756, or Anti-Biotin-APC # 130-090-856), and continue as described in step 4 and 5.
7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Example of immunofluorescent staining with CD1c (BDCA-1) antibodies

Peripheral blood leukocytes (PBLs) were stained with CD1c (BDCA-1)-APC, CD14-FITC (# 130-080-701) and 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 PI fluorescence. Note that a subset of CD19⁺ B cells expresses CD1c (BDCA-1) (upper dot plot). They are excluded from analysis in the CD14 vs. CD1c (BDCA-1) dot plot (lower dot plot). Note that expression of CD1c (BDCA-1) inversely correlates with CD14 expression.



4. References

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3. MacDonald, K. P. A. *et al.* (2002) Characterization of human blood dendritic cell subsets. *Blood* 100: 4512–4520. [2439]
4. Clark, F. J. *et al.* (2003) Origin and subset distribution of peripheral blood dendritic cells in patients with chronic graft-versus-host disease. *Transplant.* 75: 221–225. [2873]
5. Brigl, M. and Brenner, M. B. (2004) CD1: antigen presentation and T cell function. *Annu. Rev. Immunol.* 22: 817–890.
6. Lebre (2003) BDCA3^{hi} dendritic cells: a novel subset with distinct phenotypical characteristics. Doctoral dissertation, University of Amsterdam, Netherlands.
7. Peiser, M. *et al.* (2003) CD1a and CD1c cell sorting yields a homogeneous population of immature human Langerhans cells. *J. Immunol. Methods* 279: 41–53. [4298]
8. Coates, P. T. H. *et al.* (2003) Dendritic cell subsets in blood and lymphoid tissue of rhesus monkeys and their mobilization with Flt3 ligand. *Blood* 102: 2513–2521. [3099]

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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