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1. Description

Components	1 mL monoclonal CD1d antibodies, mouse conjugated to:
	FITC 130-096-338
	PE 130-096-342
	APC 130-096-332
	Biotin 130-096-302
Clone	1B1 (isotype: rat IgG2bκ).
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

Clone 1B1 reacts with CD1d also known as CD1.1 and Ly-38, a 48 kDa type I membrane glycoprotein with structural homology to MHC class I molecules. It appears to recognize CD1d only in association with β2-microglobulin. CD1d is found at varying levels on most types of bone marrow and peripheral leukocytes and on epithelial, dendritic, and lymphoid cells in the thymus.

1.2 Applications

- Identification and enumeration of CD1d⁺ cells by flow cytometry.

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD1d conjugates is **1:11 for up to 10⁷ cells/100 μL** of buffer for labeling of cells and subsequent analysis by flow cytometry.

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

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.
- (Optional) FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Anti-Biotin antibodies conjugated to, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with CD1d-Biotin.
- (Optional) CD19-FITC (# 130-092-042) or CD19-PE (# 130-092-041). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) for flow cytometric exclusion of dead cells without fixation.

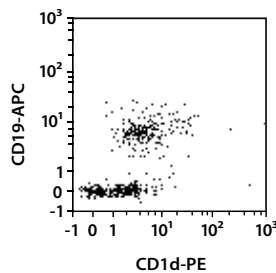
2. General protocol for immunofluorescent staining

▲ 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 μ L of buffer.
4. Add 10 μ L of the CD1d 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 CD1d-Biotin was used, resuspend the cell pellet in 100 μ L of buffer, add 10 μ L 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 CD1d antibodies

BALB/c splenocytes were stained with CD1d antibodies conjugated to PE as well as with CD19-FITC (# 130-092-042) 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.



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

4. References

1. Sydora, B. C. *et al.* (1996) TAP-independent selection of CD8+ intestinal intraepithelial lymphocytes. *J. Immunol.* 156: 4209–4216.
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4. Roark, J. H. *et al.* 1998. CD1.1 expression by mouse antigen-presenting cells and marginal zone B cells. *J. Immunol.* 160: 3121–3127.
5. Brudin, N. *et al.* (1998) Selective Ability of Mouse CD1 to Present Glycolipids: α -Galactosylceramide Specifically Stimulates Va14^+ NK T Lymphocytes. *J. Immunol* 161: 271–81.
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7. Szalay, G. *et al.* (1999) Cutting edge: anti-CD1 monoclonal antibody treatment reverses the production patterns of TGF- β 2 and Th1 cytokines and ameliorates listeriosis in mice. *J Immunol.* 162(12): 6955–6958.

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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