



Miltenyi Biotec

Anti-DCAR1 antibodies mouse

Anti-DCAR1-PE	130-095-317
Anti-DCAR1-APC	130-095-320
Anti-DCAR1-Biotin	130-095-315

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1. Description

Components	1 mL Anti-DCAR1 antibodies, mouse: monoclonal Anti-DCAR1 antibodies conjugated to R-phycoerythrin (PE), allophycocyanin (APC), or biotin.
Clone	SKa5-3D5 (isotype: rat IgG2b).
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

Dendritic cell immunoactivating receptor 1 (DCAR1) belongs to a subfamily of cell surface C-type lectin receptors (CLR). Mouse DCAR1 shows a tissue specific expression pattern: it is expressed on CD8a⁺ DC subsets in spleen and thymus and on subsets of CD11b⁺ myeloid cells in bone marrow and spleen. Mouse DCAR1 has neither been detected in lymph nodes nor in peripheral blood. Antigen delivered via anti-DCAR1 antibody is internalized and is cycled through the early and late endosomal compartment, inducing cellular and humoral responses *in vivo*. Triggering DCAR1 via monoclonal antibody ligation on CD8⁺ DCs increases the secretion of bioactive IL-12 whereas secretion of IL-10 is markedly reduced, indicating that antigen recognized by mDCAR1 induces enhanced proinflammatory responses. Anti-DCAR1 antibodies have been tested on BALB/c and C57BL/6 mice.¹

1.2 Applications

- Identification and enumeration of Anti-DCAR1⁺ cells by flow cytometry or fluorescence microscopy.

1.3 Recommended antibody dilution

For antibody labeling of mouse cells.

Anti-DCAR1 conjugate	PE	APC	Biotin
Flow cytometry ^a			
- In general	1:11	1:11	1:11
- Formaldehyde-fixed cells ^b	1:11	1:11	1:11

- a) The indicated antibody dilutions are for up to 10⁷ cells/100 µL of buffer.
b) For optimal results, cells must be stained prior to fixation.

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.
- FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Anti-Biotin-VioBlue® (# 130-094-669), 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 Anti-DCAR1-Biotin.
- (Optional) Anti-FITC MicroBeads (# 130-048-701), Anti-PE MicroBeads (# 130-048-801), Anti-APC MicroBeads (# 130-090-855), or Anti-Biotin MicroBeads (# 130-090-485) for subsequent indirect magnetic labeling.
- (Optional) CD11c-FITC (# 130-091-842). For more information about 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.

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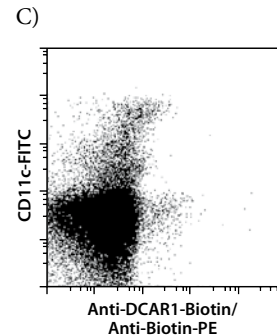
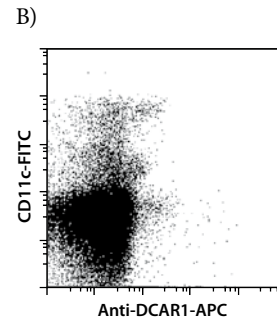
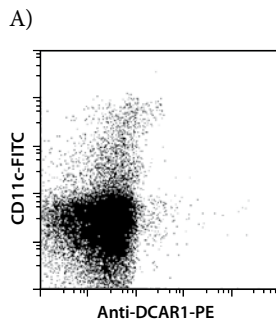
2. General protocol for immunofluorescent staining

▲ 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 90 μ L of buffer.
4. Add 10 μ L of FcR Blocking Reagent.
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. Add 10 μ L of the Anti-DCAR1 antibody.
7. 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.
8. Wash cells by adding 1–2 mL of buffer and centrifuge at $300 \times g$ for 10 minutes. Aspirate supernatant completely.
9. (Optional) If Anti-DCAR1-Biotin was used, resuspend the cell pellet in 100 μ L of buffer, add 10 μ L of anti-biotin antibody (Anti-Biotin-VioBlue, Anti-Biotin-FITC, Anti-Biotin-PE, or Anti-Biotin-APC), and continue as described in steps 7 and 8.
10. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Examples of immunofluorescent staining with Anti-DCAR1 antibodies

Mouse BALB/c splenocytes were stained with Anti-DCAR1 antibodies conjugated to PE (A) or APC (B) as well as with CD11c-FITC (# 130-091-842) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cells labeled with Anti-DCAR1-Biotin (C) were stained with Anti-Biotin-PE (# 130-090-756) as well as CD11c-FITC. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. References

1. Kaden, S. A. *et al.* (2009) Enhanced dendritic cell-induced immune responses mediated by novel C-type lectin receptor mDCAR1. *J. Immunol.* 183: 5069–5078.

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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