

# Anti-DCIR2 (33D1) antibodies

## mouse

Anti-DCIR2 (33D1)-PE	130-095-188
Anti-DCIR2 (33D1)-APC	130-095-190
Anti-DCIR2 (33D1)-Biotin	130-095-181

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## 1. Description

<b>Components</b>	1 mL Anti-DCIR2 (33D1) antibodies, mouse: monoclonal Anti-DCIR2 (33D1) antibodies conjugated to R-phycoerythrin (PE), allophycocyanin (APC), or biotin.
<b>Clone</b>	33D1 (isotype: rat IgG2b).
<b>Capacity</b>	100 tests or up to 10 <sup>9</sup> total cells.
<b>Product format</b>	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
<b>Storage</b>	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 monoclonal antibody 33D1 reacts with mouse dendritic cell inhibitory receptor 2 (DCIR2), a member of the CLEC receptor family.<sup>1-5</sup>

Anti-DCIR2 (33D1) antibody staining identifies subsets of mouse dendritic cells in lymphoid tissue including spleen, thymus, lymph nodes, and Peyer's patches distinct from mouse CD8a<sup>+</sup>CD205<sup>+</sup> DCs. DCIR2 expression by bone marrow cells is induced by treatment with GM-CSF. Binding of Anti-DCIR2 (33D1) antibodies is calcium-dependent.

### 1.2 Applications

- Identification and enumeration of DCIR2<sup>+</sup> cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Mouse DCIR2<sup>+</sup> dendritic cells can be isolated by using, for example, CD4<sup>+</sup> Dendritic Cell Isolation Kit, mouse (# 130-091-262).

### 1.3 Recommended antibody dilution

For antibody labeling of mouse cells.

Anti-DCIR2 (33D1) conjugate	PE	APC	Biotin
Flow cytometry <sup>a</sup>			
- In general	1:11	1:11	1:11
- Formaldehyde-fixed cells <sup>b</sup>	1:11	1:11	1:11

- a) The indicated antibody dilutions are for up to 10<sup>7</sup> cells/100 µL of buffer.  
 b) For optimal results, cells must be stained prior to fixation.

### 1.4 Reagent requirements

- Buffer: Prepare a phosphate-buffered solution containing Ca<sup>2+</sup> and Mg<sup>2+</sup>, pH7.2, e.g., Hanks' Balanced Salt Solution (HBSS): 138 mM NaCl, 5.3 mM KCl, 0.2 mM Na<sub>2</sub>HPO<sub>4</sub>, 0.4 mM KH<sub>2</sub>PO<sub>4</sub>, 1.3 mM CaCl<sub>2</sub>, 0.8 mM MgSO<sub>4</sub>, 4.2 mM NaHCO<sub>3</sub>. Add 0.5% bovine serum albumine (BSA). Keep buffer cold (2–8 °C).
- 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-DCIR2 (33D1)-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) or CD4<sup>+</sup> Dendritic Cell Isolation Kit, mouse (# 130-091-262). For more information about antibodies refer to [www.miltenyibiotec.com](http://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.

## 2. General protocol for immunofluorescent staining

▲ Volumes given below are for up to 10<sup>7</sup> nucleated cells. When working with fewer than 10<sup>7</sup> 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<sup>7</sup> nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ Staining of DCIR2 requires the presence of Ca<sup>2+</sup> ions. Please use the recommended buffer for optimal performance of the antibody.

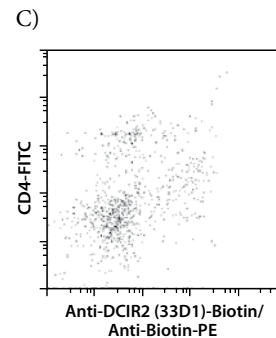
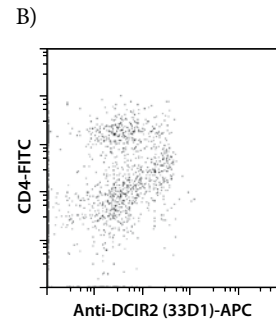
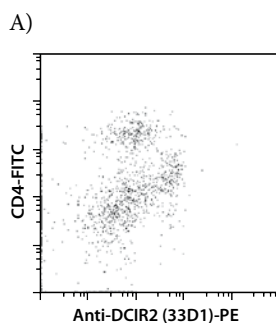
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10<sup>7</sup> nucleated cells per 90 μL of buffer.
4. Add 10 μL of FcR Blocking Reagent.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
 

▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
6. Add 10 μL of the Anti-DCIR2 (33D1) antibody.
7. 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.
8. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
9. (Optional) If Anti-DCIR2 (33D1)-Biotin was used, resuspend the cell pellet in 100 μL of buffer, add 10 μL 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-DCIR2 (33D1) antibodies

Mouse C57BL/6 splenocytes were stained with CD4-FITC (# 130-091-608) and also stained with Anti-DCIR2 (33D1) antibodies conjugated to PE (A) or APC (B) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cells labeled with Anti-DCIR2 (33D1)-Biotin (C) were stained with Anti-Biotin-PE (# 130-090-756). Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence. Cells were gated on CD11c<sup>high</sup>.



### 4. References

1. Nussenzweig, M. C. *et al.* (1982) A monoclonal antibody specific for mouse dendritic cells. *Proc. Natl. Acad. Sci. USA* 79: 161–165.
2. Vremec, D. *et al.* (1992) The surface phenotype of dendritic cells purified from mouse thymus and spleen: investigation of the CD8 expression by a subpopulation of dendritic cells. *J. Exp. Med.* 176: 47–58.
3. Masurier, C. *et al.* (1999) Immunophenotypical and functional heterogeneity of dendritic cells generated from murine bone marrow cultured with different cytokine combinations: implications for anti-tumoral cell therapy. *Immunol.* 96: 569–577.
4. Dudziak, D. *et al.* (2007) Differential antigen processing by dendritic cell subsets in vivo. *Science* 315: 107–111.
5. Geijtenbeek, T. B. (2004) Self- and nonself-recognition by C-type lectins on dendritic cells. *Annu. Rev. Immunol.* 22: 33–54.

All protocols and data sheets are available at [www.miltenyibiotec.com](http://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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