Anti-DCIR2 (33D1) antibodies, mouse

For research use only

9 µg equal 60 tests, 30 µg equal 200 tests. One test corresponds to labeling of 10^6 cells.

Product	Content	Order no.
Anti-DCIR2 (33D1)-PE	30 µg in 1 mL	130-102-188
Anti-DCIR2 (33D1)-APC	30 µg in 1 mL	130-102-177
Anti-DCIR2 (33D1)-Biotin	30 µg in 1 mL	130-101-843

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.

Technical data and background information

Antigen	DCIR2 (33D1)
Clone	33D1
Isotype	rat IgG2bκ
Isotype control	Rat IgG2b – isotype control antibodies
Alternative names of antigen	CLEC4A4
Molecular mass of antigen [kDa]	27
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	Cells should be stained prior to fixation, if formaldehyde is used as a fixative.
Storage	Store protected from light at 2–8 °C. Do not freeze.

The monoclonal antibody 33D1 reacts with mouse dendritic cell inhibitory receptor 2 (DCIR2), a member of the CLEC receptor family. 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 $CD8a^+CD205^+$ DCs. DCIR2 expression by bone marrow cells is induced by treatment with GM-CSF. Binding of Anti-DCIR2 (33D1) antibodies is calcium-dependent.

Reagent requirements

- Prepare a solution containing Ca^{2+} and Mg^{2+}, pH 7.2; e.g. Hanks' Balanced Salt Solution (HBSS): 138 mM NaCl, 5.3 mM KCl, 0.2 mM Na_2HPO_4, 0.4 mM KH_2PO_4, 1.3 mM $CaCl_2$, 0.8 m M $MgSO_4$, 4.2 mM $NaHCO_3$. Add 0.5 % bovine serum albumine (BSA). Keep buffer cold (2−8 °C).
- (Optional) FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor–mediated antibody labeling.

- (Optional) Fluorochrome-conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with biotinylated 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.

Protocol for cell surface staining

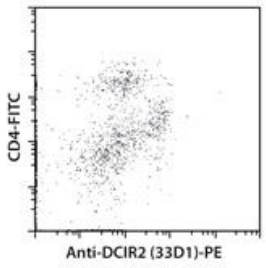
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:10 for up to 10^6 cells/50 µL of buffer.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^6 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 45 µL of buffer.
4. Add 5 µL of the antibody.
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. Wash cells by adding 1−2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) If biotinylated antibody was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of fluorochrome-conjugated 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.

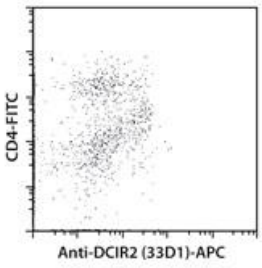
Examples of immunofluorescent staining

Mouse C57BL/6 splenocytes were stained with CD4-FITC 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. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence. Cells were gated on $CD11c^{high}$.

A:



B:



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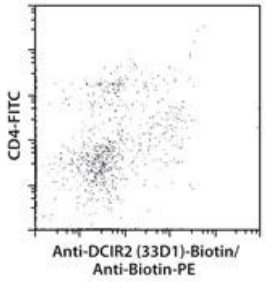

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