

CD336 (NKp44) antibodies, human

For research use only

One test corresponds to labeling of up to 10^7 cells in a total volume of 100 μ L.

| Product | Content | Order no. |
|------------------------------|---------------------|-------------|
| CD336 (NKp44)-VioBright FITC | for 30 tests | 130-105-037 |
| CD336 (NKp44)-VioBright FITC | for 100 tests | 130-104-985 |
| CD336 (NKp44)-PE | for 30 tests | 130-099-684 |
| CD336 (NKp44)-PE | for 100 tests | 130-092-480 |
| CD336 (NKp44)-APC | for 30 tests | 130-099-414 |
| CD336 (NKp44)-APC | for 100 tests | 130-092-481 |
| CD336 (NKp44)-PE-Vio770 | for 30 tests | 130-104-228 |
| CD336 (NKp44)-PE-Vio770 | for 100 tests | 130-104-195 |
| CD336 (NKp44)-Biotin | for 30 tests | 130-100-091 |
| CD336 (NKp44)-Biotin | for 100 tests | 130-092-482 |
| CD336 (NKp44) pure | 100 μ g in 1 mL | 130-092-556 |

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 | CD336 (NKp44) |
| Clone | 2.29 |
| Isotype | mouse IgG1 |
| Isotype control | Mouse IgG1 – isotype control antibodies |
| Alternative names of antigen | NCR2, LY95, NKp44 |
| Molecular mass of antigen [kDa] | 28 |
| Distribution of antigen | NK cells, T cells |
| 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. |

Clone 2.29 recognizes the CD336 antigen, also known as NKp44. CD336 is a member of the natural cytotoxicity receptor (NCR) family which trigger cytotoxicity in NK cells. CD336 is directly involved in target cell recognition and lysis and is only expressed by activated NK cells, e.g., NK cells cultured in the

presence of IL-2. Freshly isolated, resting NK cells do not express CD336 (Nkp44).

Reagent requirements

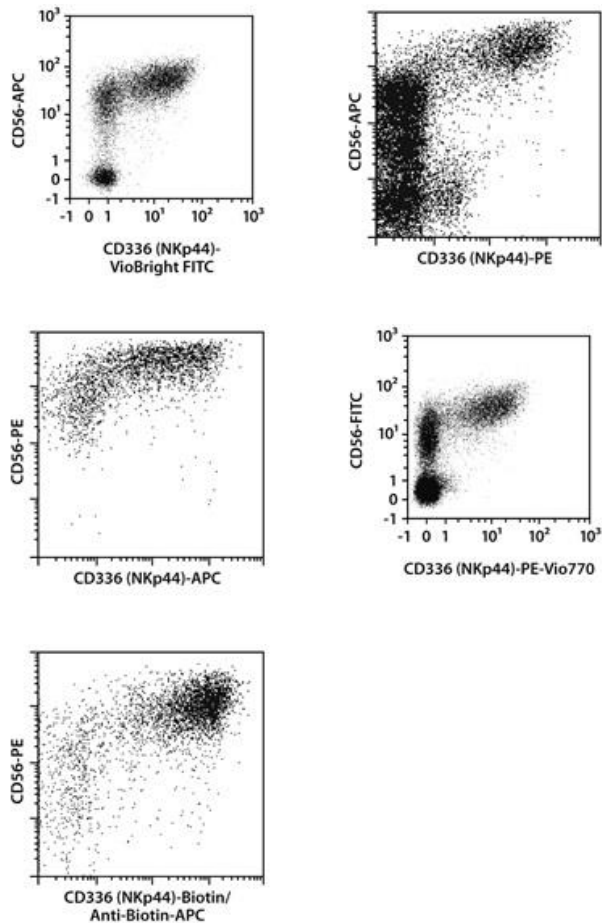
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS[®] BSA Stock Solution (# 130-091-376) 1:20 with autoMACS[®] Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).
Note: EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) 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 1:11 for up to 10⁷ cells/100 µL of buffer.
 - Volumes given below are for up to 10⁷ nucleated cells. When working with fewer than 10⁷ 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 2×10⁷ 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 10⁷ nucleated cells per 100 µL of buffer.
 4. Add 10 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

Human NK cells were activated with IL-2 and autologous monocytes for 3 days. Cells were stained with CD336 (Nkp44) antibodies as well as CD56 antibodies and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



References

1. **Vitale, M. et al.** (1998) NKp44, a novel triggering surface molecule specifically expressed by activated natural killer cells, is involved in non-major histocompatibility complex-restricted tumor cell lysis. *J. Exp. Med.* 187: 2065–2072.
2. **Sivori, S. et al.** (2000) 2B4 functions as a co-receptor in human NK cell activation. *Eur. J. Immunol.* 30: 787–793.
3. **Moretta, A. et al.** (2001) Activating receptors and coreceptors involved in human natural killer cell-mediated cytotoxicity. *Annu. Rev. Immunol.* 19: 197–223.
4. **Moretta, L. and Moretta, A.** (2004) Unravelling natural killer function: Triggering and inhibitory human NK receptors. *EMBO J.* 23: 255–259.
5. **Chaput, N. et al.** (2013) Phase I clinical trial combining imatinib mesylate and IL-2: HLA-DR(+) NK cell levels correlate with disease outcome. *Oncoimmunology* 2(2): e23080.

Warranty

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