Miltenyi Biotec

CD337 (NKp30) antibodies, human

For research use only

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

Product	Content	Order no.
CD337 (NKp30)-PE	for 30 tests	130-099-706
CD337 (NKp30)-PE	for 100 tests	130-092-483
CD337 (NKp30)-APC	for 30 tests	130-099-380
CD337 (NKp30)-APC	for 100 tests	130-092-484
CD337 (NKp30)-PE-Vio770	for 30 tests	130-104-154
CD337 (NKp30)-PE-Vio770	for 100 tests	130-104-116
CD337 (NKp30)-Biotin	for 100 tests	130-092-553
CD337 (NKp30) pure	100 µg in 1 mL	130-092-554

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	CD337 (NKp30)
Clone	AF29-4D12
Isotype	mouse IgG1κ
Isotype control	Mouse IgG1 – isotype control antibodies
Alternative names of antigen	NCR3, 1C7, LY117, MALS, NKp30
Molecular mass of antigen [kDa]	20
Cross-reactivity	rhesus monkey (*Macaca mulatta*), cynomolgus monkey (*Macaca fascicularis*)
Distribution of antigen	NK 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.

The CD337 (NKp30) receptor is a 30 kDa type I membrane glycoprotein characterized by a single V-type Ig-like domain in the extracellular portion, and is found almost exclusively on the surface of resting or activated natural killer (NK) cells. CD337 is a member of the natural cytotoxicity receptor (NCR) family which trigger cytotoxicity in NK cells. CD337 is directly involved in target cell recognition and lysis and is expressed by all resting or activated NK cells and by a small subset of T cells. Its expression density

parallels that of CD336 (NKp44). It seems to be involved in killing of cells which are lysed independently from CD336 (NKp44) and CD335 (NKp46).

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 Ca^{2+} or Mg^{2+} 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.

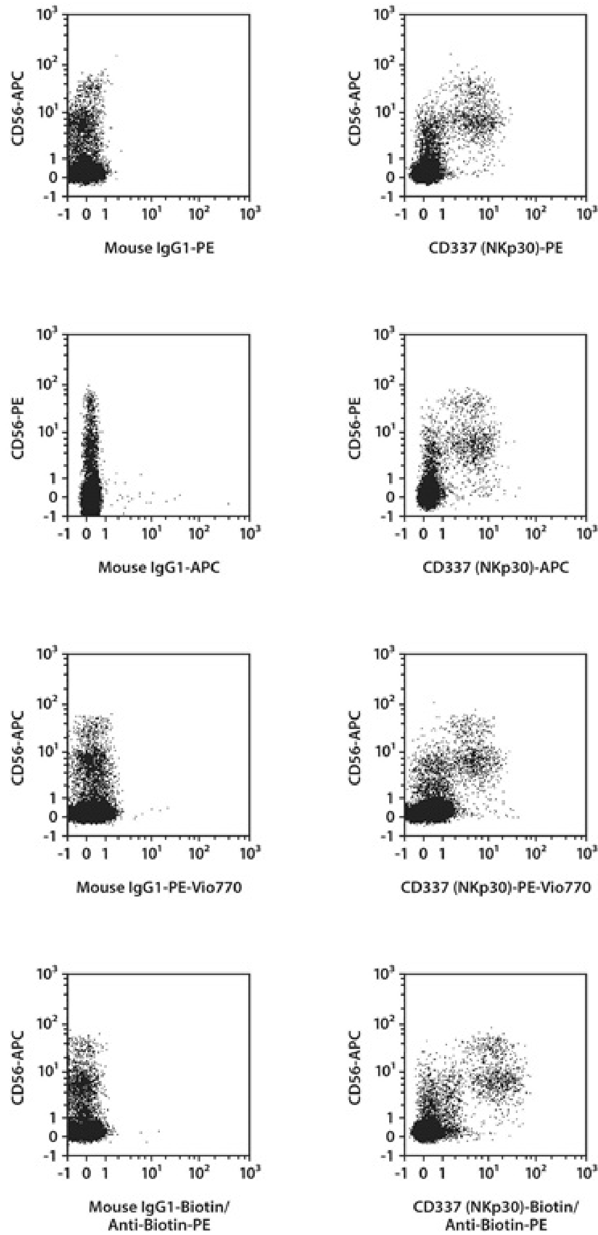
Protocol for cell surface staining

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 for up to 10^7 cells/100 µL of buffer.
- 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×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 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.

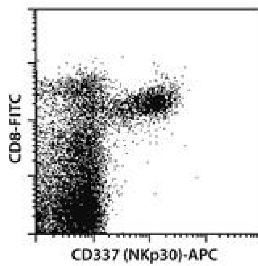
Examples of immunofluorescent staining

Human peripheral blood mononuclear cells (PBMCs) were stained with CD337 (NKp30) antibodies or with the corresponding isotype control antibodies (left image) as well as with CD56 antibodies, and analyzed by flow cytometry using the MACSQuant® Analyzer. The Tandem Signal Enhancer has been used to increase binding specificity of tandem-dye–conjugated antibodies. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandems.

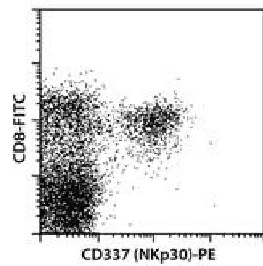


Rhesus monkey (A) or cynomolgus monkey (B) PBMCs were stained with CD337 (NKp30)-APC or -PE, respectively, and CD8-FITC. Cells were analyzed by flow cytometry.

A:



B:



References

1. **Pende, D. *et al.*** (1999) Identification and molecular characterization of Nkp30, a novel triggering receptor involved in natural cytotoxicity mediated by human natural killer cells. J. Exp. Med. 190: 1505–1516.
2. **Moretta, L. and Moretta, A.** (2004) Unravelling natural killer function: Triggering and inhibitory human NK receptors. EMBO J. 23: 255–259.
3. **Sivori, S. *et al.*** (2000) 2B4 functions as a co-receptor in human NK cell activation. Eur. J. Immunol. 30: 787–793.

4. **Moretta, A. *et al.*** (2001) Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis. Annu. Rev. Immunol. 19: 197–223.

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