

Anti-NKp46 antibodies, mouse

For research use only

9 µg equal 60 tests, 30 µg equal 200 tests. One test corresponds to labeling of 10⁶ cells.

Product	Content	Order no.
Anti-NKp46-FITC	9 µg in 300 µL	130-102-842
Anti-NKp46-FITC	30 µg in 1 mL	130-102-300
Anti-NKp46-PE	9 µg in 300 µL	130-102-821
Anti-NKp46-PE	30 µg in 1 mL	130-102-395
Anti-NKp46-APC	9 µg in 300 µL	130-102-823
Anti-NKp46-APC	30 µg in 1 mL	130-102-347
Anti-NKp46-VioBlue	9 µg in 300 µL	130-103-137
Anti-NKp46-VioBlue	30 µg in 1 mL	130-102-185
Anti-NKp46-Biotin	9 µg in 300 µL	130-101-993
Anti-NKp46-Biotin	30 µg in 1 mL	130-101-886
Anti-NKp46 pure	100 µg in 1 mL	130-095-118

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	NKp46
Clone	29A1.4.9
Isotype	rat IgG2ak
Isotype control	Rat IgG2a – isotype control antibodies
Alternative names of antigen	NCR1, LY94, CD335, MAR-1
Molecular mass of antigen [kDa]	36
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.

NKp46 is a type I transmembrane protein with two extracellular Ig-like domains. It is a member of the natural cytotoxicity receptor (NCR) family, which triggers cytotoxicity in NK cells. NKp46 is involved in target cell recognition and lysis and seems to be exclusively expressed on NK cells. Staining has been

shown on BALB/c, SJL, CBA/CA, C57Bl/6, NOD, DBA/2, and B6.129 mice.¹

Reagent requirements

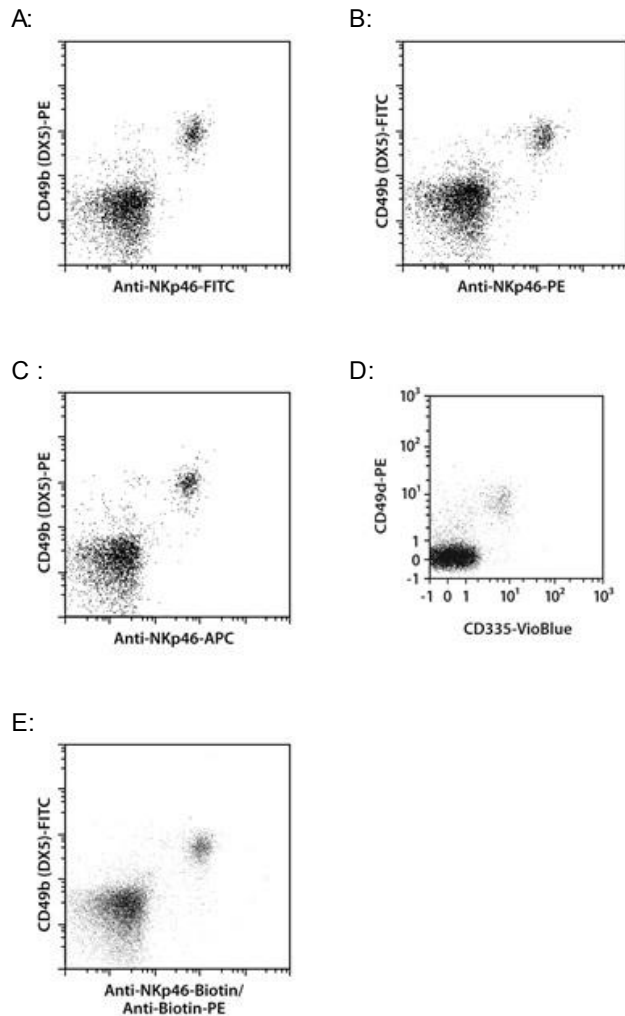
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS[®] BSA Stock Solution (# 130-091-376) 1:20 with autoMACS[®] Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).
Note: EDTA can be replaced by other 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, 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 10⁶ cells/50 µL of buffer.
 - Volumes given below are for up to 10⁶ nucleated cells. When working with fewer than 10⁶ 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 2×10⁶ 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 10⁶ 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 splenocytes were stained with Anti-NKp46 antibodies conjugated to FITC (A), PE (B), APC (C), VioBlue (D) or Biotin (E) as well as with CD49b (DX5)-FITC or CD49b (DX5)-PE and analyzed by flow cytometry using the MACSQuant[®] Analyzer. Cells stained with Anti-NKp46-Biotin were stained with Anti-Biotin-PE in addition. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



References

1. Walzer, T. *et al.* (2007) Proc. Natl. Acad. Sci. U.S.A. 104: 3384–3389.

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