

# Anti-NKp46 antibodies

## mouse

Anti-NKp46-FITC	130-095-115
Anti-NKp46-PE	130-095-116
Anti-NKp46-APC	130-095-119
Anti-NKp46-Biotin	130-095-117
Anti-NKp46 pure	130-095-118

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## 1. Description

<b>Components</b>	1 mL Anti-NKp46 antibodies, mouse: monoclonal Anti-NKp46 antibodies conjugated to fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), allophycocyanin (APC), or biotin. The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL.
<b>Clone</b>	29A1.4.9 (isotype: rat IgG2a).
<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

Clone 29A1.4.9 recognizes mouse NKp46, also known as MAR-1 or CD335, 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 is exclusively expressed on NK cells, suggesting it to be a universal NK cell marker in the mouse. Staining has been shown on BALB/C, SJL, CBA/CA, C57Bl/6, NOD, DBA/2, and B6.129 mice.<sup>1</sup>

### 1.2 Applications

- Identification and enumeration of NKp46<sup>+</sup> cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy.

### 1.3 Recommended antibody dilution

For antibody labeling of mouse cells.

Anti-NKp46 conjugate	FITC	PE	APC	Biotin
Flow cytometry <sup>a</sup>				
- In general	1:11	1:11	1:11	1:11
- Formaldehyde-fixed cells <sup>b</sup>	1:11	1:11	1:11	1:11
Immunohistochemistry <sup>c</sup>				n. r.

- 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.  
 c) The optimal antibody dilution should be determined by the user.

### 1.4 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). BSA can be replaced by other proteins such as mouse serum albumin, mouse serum, or fetal bovine serum. Buffers or media containing Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.
- (Optional) 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-NKp46-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) CD49b (DX5)-FITC (# 130-091-814) or CD49b (DX5)-PE (# 130-091-816). 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.

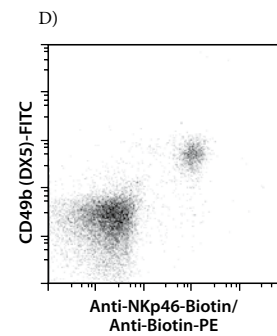
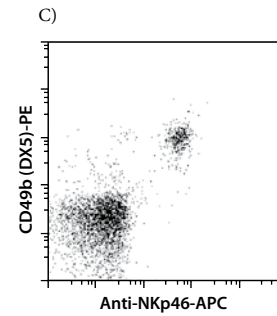
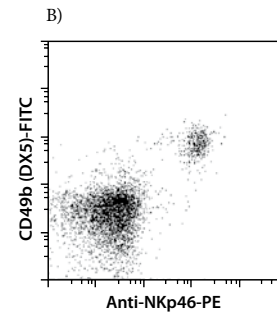
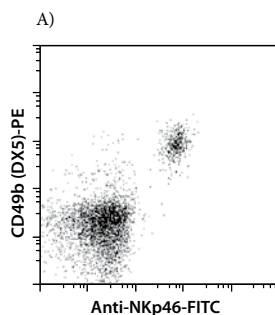
## 2. General protocol for immunofluorescent staining

▲ Volumes given below are for up to  $10^7$  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  $2 \times 10^7$  nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to  $10^7$  nucleated cells per 100  $\mu\text{L}$  of buffer.
4. Add 10  $\mu\text{L}$  of the Anti-NKp46 antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator ( $2-8^\circ\text{C}$ ).  
▲ **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  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
7. (Optional) If Anti-NKp46-Biotin was used, resuspend the cell pellet in 100  $\mu\text{L}$  of buffer, add 10  $\mu\text{L}$  of anti-biotin antibody (Anti-Biotin-FITC, Anti-Biotin-PE, or Anti-Biotin-APC), 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.

## 3. Examples of immunofluorescent staining with Anti-NKp46 antibodies

Mouse splenocytes were stained with Anti-NKp46 antibodies conjugated to FITC (A), PE (B), or APC (C), as well as with CD49b (DX5)-FITC (# 130-091-814) or CD49b (DX5)-PE (# 130-091-816) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cells labeled with Anti-NKp46-Biotin (D) were stained with Anti-Biotin-PE (# 130-090-756) as well as CD49b (DX5)-FITC (# 130-091-814). Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



## 4. Reference

1. Walzer, T. *et al.* (2007) Identification, activation, and selective *in vivo* ablation of mouse NK cells via NKp46. *Proc. Natl. Acad. Sci.* 104: 3384–3389.

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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