

# Anti-NKp80 antibodies human

Anti-NKp80-FITC	130-094-843
Anti-NKp80-PE	130-094-844
Anti-NKp80-APC	130-094-845
Anti-NKp80-Biotin	130-095-114
Anti-NKp80 pure	130-094-846

## Contents

1. Description
  - 1.1 Background information
  - 1.2 Applications
  - 1.3 Recommended antibody dilution
  - 1.4 Reagent requirements
2. General protocol for immunofluorescent staining
3. Examples of immunofluorescent staining with Anti-NKp80 antibodies
4. References

## 1. Description

<b>Components</b>	1 mL Anti-NKp80 antibodies, human: monoclonal Anti-NKp80 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>	4A4.D10 (isotype: mouse IgG1).
<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

NKp80, also termed KLRF1, is an 80 kDa homodimeric surface molecule and belongs to the family of C-type lectin-like receptors. It is expressed on all human natural killer (NK) cells and a small subset of effector memory CD8 T cells with an inflammatory NK-like phenotype.<sup>1,2</sup>

NKp80 stimulates NK cell cytotoxicity and cytokine release and appears to cooperate with other triggering receptors to induce optimal NK cell activation upon interaction with potential target cells.<sup>1</sup> The ligand of NKp80 is AICL (activation-induced C-type lectin), also called CLEC2B. NKp80 engagement by AICL is discussed to promote cytolysis of myeloid cells and to be involved in the mutual activation of NK cells and monocytes.<sup>3</sup>

### 1.2 Applications

- Identification and enumeration of Anti-NKp80<sup>+</sup> cells by flow cytometry or fluorescence microscopy.

### 1.3 Recommended antibody dilution

For antibody labeling of human cells.

Anti-NKp80 conjugate	FITC	PE	APC	Biotin
<b>Flow cytometry<sup>a</sup></b>				
- In general	1:11	1:11	1:11	1:11
- Formaldehyde-fixed cells <sup>b</sup>	1:11	1:11	1:11	1:11

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.

- Cross-reactivity: The Anti-NKp80 antibody is tested to react with rhesus monkey (*Macaca mulatta*) and cynomolgus monkey (*Macaca fascicularis*) cells.

### 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<sup>®</sup> BSA Stock Solution (# 130-091-376) 1:20 with autoMACS<sup>®</sup> 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 human serum albumin, human serum, or fetal bovine serum. Buffers or media containing Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (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-NKp80-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) CD56-PE (# 130-090-755) or CD56-APC (# 130-090-843). For more information about fluorochrome-conjugated antibodies see [www.miltenyibiotec.com](http://www.miltenyibiotec.com).
- (Optional) Mouse IgG1-FITC (# 130-092-213), Mouse IgG1-PE (# 130-092-212), or Mouse IgG1-APC (# 130-092-214) for isotype control.
- (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<sup>7</sup> nucleated cells. When working with fewer than 10<sup>7</sup> 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).

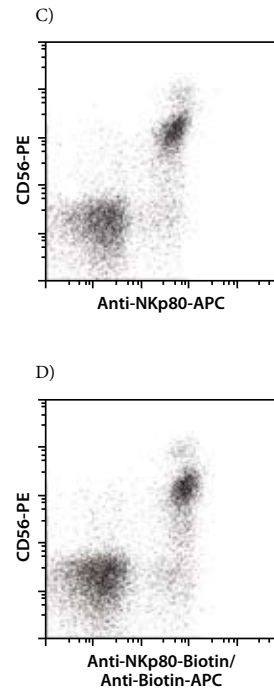
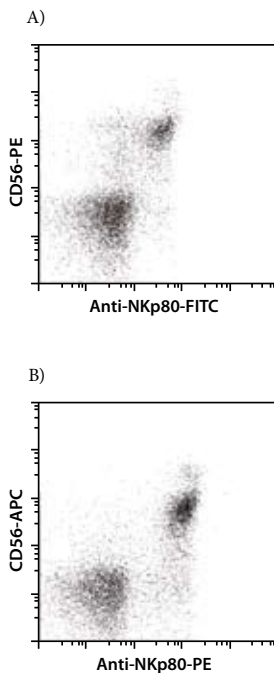
1. Determine cell number.
2. Centrifuge cell suspension at  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to  $10^7$  nucleated cells per 100  $\mu\text{L}$  of buffer.
4. Add 10  $\mu\text{L}$  of the Anti-NKp80 antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator ( $2-8^\circ\text{C}$ ).

▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

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-NKp80-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-NKp80 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with Anti-NKp80 antibodies conjugated to FITC (A), PE (B), or APC (C), as well as with CD56-PE (# 130-090-755) and CD56-APC (# 130-090-843) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cells labeled with Anti-NKp80-Biotin (D) were stained with Anti-Biotin-APC (# 130-090-856) as well as CD56-PE. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



### 4. References

1. Vitale, M. *et al.* (2001) Identification of NKp80, a novel triggering molecule expressed by human NK cells. *Eur. J. Immunol.* 31: 233–242.
2. Kuttruff, S. *et al.* (2009) NKp80 defines and stimulates a reactive subset of CD8 T cells. *Blood* 113: 358–369.
3. Welte, S. *et al.* (2006) Mutual activation of natural killer cells and monocytes mediated by NKp80-AICL interaction. *Nat. Immunol.* 7: 1334–1342.

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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