

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-NK1.1 antibodies
4. References

1. Description

Components	1 mL monoclonal Anti-NK1.1 antibodies, mouse, conjugated to various dyes.						
	<table border="0"> <tr> <td>PE</td> <td>130-095-867</td> </tr> <tr> <td>APC</td> <td>130-095-869</td> </tr> <tr> <td>Biotin</td> <td>130-095-865</td> </tr> </table>	PE	130-095-867	APC	130-095-869	Biotin	130-095-865
PE	130-095-867						
APC	130-095-869						
Biotin	130-095-865						
Clone	PK136 (isotype: mouse IgG2a).						
Capacity	100 tests or up to 10 ⁹ total cells.						
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.						
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.						

1.1 Background information

The PK136 monoclonal antibody reacts with NK1.1, also known as CD161. NK1.1 is expressed by natural killer cells and a subset of T cells in the mouse strains C57BL/6, FVB/N, and NZB, but not in the mouse strains AKR, BALB/c, CBA/J, C3H, DBA/1, DBA/2, NOD, SJL, and 129.

Clone PK136 has been reported to deplete NK cells, induce redirected lysis, block NK cell function, and induce NK cell proliferation.^{1–6}

1.2 Applications

- Identification and enumeration of NK1.1⁺ cells by flow cytometry or fluorescence microscopy.

1.3 Recommended antibody dilution

The recommended antibody dilution for all Anti-NK1.1 conjugates is **1:11 for up to 10⁷ cells/100 μL** of buffer for labeling of cells and analysis by flow cytometry.

The antibody is suited for staining of formaldehyd-fixed cells.

Cells should be stained prior to fixation, if formaldehyde is used as a fixative.

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 (FBS). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) Anti-Biotin antibodies conjugated to, e.g., PE (# 130-090-756) as secondary antibody reagent in combination with Anti-NK1.1-Biotin.
- (Optional) Anti-NKp46-FITC (# 130-095-115). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Mouse IgG2a isotype control antibodies conjugated to, e.g., PE (# 130-091-835). For more information about isotype control antibodies refer to 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

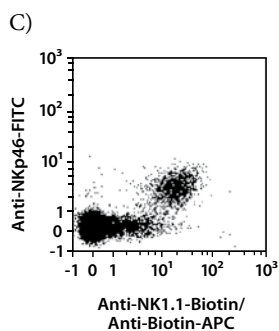
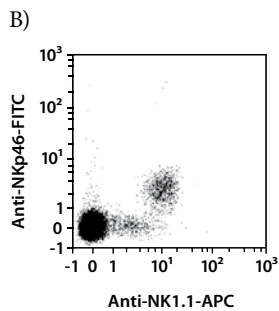
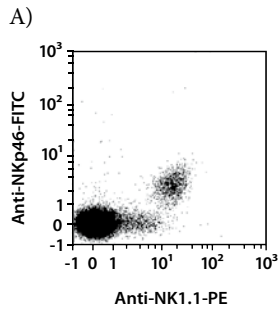
▲ Volumes given below are for up to 10⁷ nucleated cells. When working with fewer than 10⁷ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10⁷ nucleated cells, use twice the volume of all indicated 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 Anti-NK1.1 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 Anti-NK1.1-Biotin was used, resuspend the cell pellet in 100 μ L of buffer, add 10 μ L of 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.

3. Examples of immunofluorescent staining with Anti-NK1.1 antibodies

Spleen cells from C57BL/6 mice were fluorescently stained with NK1.1 antibodies conjugated to PE (A) or APC (B) as well as with Anti-NKp46-FITC (# 130-095-115) and analyzed by flow cytometry using the MACSQuant[®] Analyzer. Cells labeled with Anti-NK1.1-Biotin (C) were stained with Anti-Biotin-APC (# 130-090-856) as well as Anti-NKp46-FITC. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. References

1. Koo, G. and Peppard, J. (1984) Establishment of monoclonal Anti NK1.1 antibody. *Hybridoma*. 3: 301-303.
2. Koo, G. *et al.* (1986) The NK1.1(-) mouse: A model to study differentiation of murine NK cells. *J. Immunol.* 137: 3742-3747.
3. Karlhofer, F. *et al.* (1991) Stimulation of murine NK cells by a monoclonal antibody specific for the NK1.1 antigen. *J. Immunol.* 146: 3662-3673.
4. Kung, S. and Miler, R. (1995) The NK1.1 antigen in NK-mediated F1, antiparent killing *in vitro*. *J. Immunol.* 154: 1624-1633.
5. Carlyle, J. *et al.* (1999) Mouse NKR-P1B, a novel NK1.1 antigen with inhibitory function. *J. Immunol.* 162: 5917-5923.
6. Kung, S. *et al.* (1999) The NKR-P1B gene product is an inhibitory receptor on SJL/J NK cells. *J. Immunol.* 162: 5876-5887.

All protocols and data sheets are available at 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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