

For Research Use Only. Not For Use In Diagnostic Procedures.

SPECIFICITY

Natural Killer (NK) cells are innate immune effectors: they can exert natural cytotoxicity and secrete cytokines and chemokines in the absence of sensitization (1). NK cells also mediate antibody-dependent cellular cytotoxicity (ADCC) via Fc γ RIII (CD16). Via multiple receptors, NK cells can select (with Natural Killer Receptors: NKR) and lyse (with NKG2D, and Natural Cytotoxicity Receptors: NCR) a variety of target cells, as reviewed in Ref. 2. Unlike NKR, NCRs are strictly restricted to NK cells. To date, and beside many existing coreceptors, 3 NCRs have been identified: NKp46, NKp44 and NKp30. Their common features are (1):

- a direct involvement in target cell recognition and killing;
- the delivery of activating signals (3) by association with ITAM-containing molecules (KARAP / DAP12, or CD3 ζ , or Fc ϵ R1 γ);
- they belong to the Immunoglobulin superfamily;
- unknown ligands;
- their surface density varies in different individuals and also in the NK cells isolated from a given individual;
- there is a direct correlation between their surface density and the ability of NK cells to kill various target cells;
- their collective blocking with monoclonal antibodies strongly inhibits NK-mediated cytotoxicity.

The discovery of NKp46 resulted from the selection of monoclonal antibodies that triggered NK-mediated target cell lysis (4). BAB281 was selected on these criteria, and helped identify NKp46, a 46 kDa trans-membrane glycoprotein, strictly expressed by all NK cells, including the infrequent CD3⁺CD56⁺CD16⁻ subset (4).

The molecular cloning of NKp46 (5), and biochemical studies (6), revealed that NKp46 is associated with the ITAM-containing CD3 ζ , although the NKp46 expression on the cell surface does not require that of CD3 ζ . The surface density of NKp46 is linked to the cytolytic activity of NK cells (7). NKp46, as NKp30, is involved in the interaction of NK cells with autologous antigen-presenting cells (8). Recent findings illustrate the role of NKp46 in NK cytotoxic mechanisms in response to intracellular infection: microbial ligands for NKp46 have been identified: hemagglutinin of the influenza virus, and hemagglutinin of the Sendai virus (9). In addition, it was recently reported that freshly isolated NK cells from healthy subjects can lyse Mycobacterium tuberculosis-infected monocytes, and that the lysis is abolished by an anti-NKp46 antiserum. In tuberculosis patients, the NK cell lytic activity was reduced (10).

Beyond its role in triggering natural cytotoxicity, the cellular distribution of NKp46

is noteworthy: on freshly isolated PBMCs, NKp46 is strictly restricted to CD3⁺CD56⁺ cells, including CD56^{bright}/CD16⁻; in addition, NKp46 is not expressed by the rare CD16⁺CD56⁺ T cells. These data indicate that NKp46 may be a "all NK / only NK"-marker (4). A weak expression of NKp46 has been described on MZ93 (11) and NK-92 (12) cell lines.

The monoclonal antibody BAB281 was first studied on human NK cells (4) and exhibits the following properties:

- BAB281 reacts with all and only mature NK cells, also in Macaques (13);
- BAB281 binding to NKp46 induces a strong increase of cytolytic activity in redirected killing assays, also in Macaque (13);
- this triggering was also observed on CD16⁻ NK cells, which did not respond to anti-CD16 antibodies;
- the NKp46-induced triggering was abolished by the simultaneous cross-linking of KIRs and CD94 / NKG2A (4);
- BAB281 binding to NKp46 induces mobilization of intracellular Calcium;
- BAB281 masking of NKp46 induces a strong inhibition of NK-mediated natural cytotoxicity against HLA-Class I-negative or autologous cells covered with an anti-HLA-Class I antibody (7).

REAGENT

IOTest NKp46-PE Conjugated Antibodies
PN IM3711 – 50 tests – 20 µL / test

Clone BAB281
Isotype IgG1, mouse
Immunogen Human NK clone (SE192)
Source Ascites fluid
Purification Ion exchange or affinity chromatography
Conjugation R-phycoerythrin (PE) is conjugated at 0.5 – 1.5 moles of PE per mole of Ig.

Excitation wavelength: 488 nm
Maximum emission wavelength: 575 nm
Main emission color: Orange-red

Buffer 2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide.

APPLICATION

Flow Cytometry (4, 5, 7, 11, 13 – 15).

STATEMENT OF WARNINGS

1. This reagent contains 0.1% sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.
2. Specimens, samples and all material coming in contact with them should be

handled as if capable of transmitting infection and disposed of with proper precautions.

3. Never pipet by mouth and avoid contact of samples with skin and mucous membranes
4. Do not use antibody beyond the expiration date on the label.
5. Do not expose reagents to strong light during storage or incubation.
6. Avoid microbial contamination of reagents or incorrect results might occur.

STORAGE CONDITIONS AND STABILITY

This reagent is stable up to the expiration date when stored at 2 – 8°C. Do not freeze. Minimize exposure to light.

REAGENT PREPARATION

No reconstitution is necessary. This monoclonal antibody may be used directly from the vial. Bring reagent to 18 – 25°C prior to use.

PROCEDURE

Preparation of working solutions (quantity for 1 tube):

- 1) "Fix-and-lyse" mixture: by freshly mixing 1 mL of VersaLyse (PN IM3648) with 25 µL of undiluted IOTest 3 Fixative Solution (PN IM3515). Prepare a sufficient amount of the "fix-and-lyse" mixture for the total number of samples.
- 2) Fixing buffer: by mixing 6.25 µL of undiluted IOTest 3 Fixative Solution (PN IM3515) in 0.5 mL PBS. Prepare a sufficient amount of the fixing buffer for the total number of samples.

NOTE: Unlike what is stated on the package insert of the the IOTest 3 Fixative Solution (PN IM3515), the present procedure does not use this fixative solution as a 10X concentrated solution.

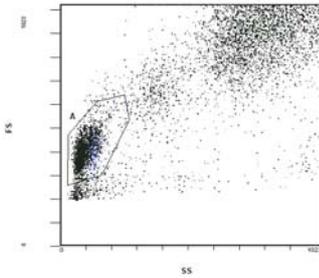
Procedure:

1. Label tubes for analysis.
2. Pipet into each tube 20µL of the monoclonal antibody (mAb) or mAb mixture.
3. Add 100 µL of whole blood.
4. Vortex each tube for 5 seconds.
5. Incubate at room temperature (18 – 25°C) for 20 minutes. Protect from light.
6. Add 1 mL of the "fix-and-lyse" mixture to each tube and vortex immediately for one second after each addition.
7. Incubate at room temperature for at least 10 minutes. Let tubes sit, protected from light.
8. Centrifuge the tubes at 150 x g for 5 minutes and discard the supernatant by aspiration.
9. Add 3 mL of PBS.
10. Centrifuge the tubes at 150 x g for 5 minutes and discard the supernatant by aspiration.
11. Resuspend the pellets by addition of 0.5 mL of fixing buffer.

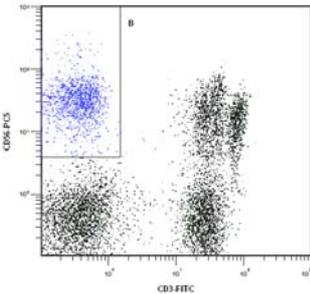
12. Vortex each tube for 5 seconds.
13. Store at 2 – 8°C until analysis:
 - a) for fresh specimens (<12 hours), analyze within 6 hours;
 - b) for older specimens (>12 hours), analyze within 2 hours.

EXAMPLE DATA

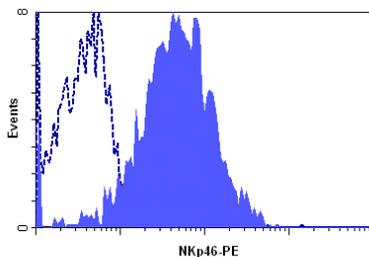
The graphs below illustrate the strategy used to study NK receptors on NK cells. They were obtained on normal whole blood samples labeled with CD3-FITC / CD56-PC5 and NKp46-PE, and lysed according to the procedure described above.



Histogram 1: FS versus SS, shows lymphocytes, monocytes and granulocytes. An amorphous region A is drawn around lymphocytes.



Histogram 2: CD56-PC5 versus CD3-FITC, gated on lymphocytes (region A), represents the expression of both the CD56 and the CD3 on lymphocytes. A rectilinear region B is set around the CD3⁺CD56⁺ lymphocytes which represents the NK cells.



Histogram 3: is a single parameter histogram of NKp46-PE, gated on regions A and B. It represents NKp46 expression on NK cells.

Isotypic Control (PN IM0670) labeling is shown underneath in light. Acquisition is with a COULTER[®] EPICS[®] XL[™] flow cytometer. Analysis is with the Beckman Coulter Expo32[™] software.

SELECTED RESEARCH REFERENCES

1. [5693] Moretta, A., Bottino, C., Vitale, M., Pende, D., Cantoni, C., Mingari, M.C., Biassoni, R., Moretta, L., "Activating receptors and coreceptors involved in human natural killer cell-mediated cytotoxicity", 2001, *Annu. Rev. Immunol.*, 19, 197-223.
2. [5722] Farag, S.S., Fehniger, T.A., Ruggeri, L., Velardi, A., Caligiuri, M.A., "Natural Killer cell receptors: new biology and insights into the graft-versus-leukemia effect", 2002, *Blood*, 6, 100, 1935-1947.
3. [5701] Tomasello, E., Bléry, M., Vély, F., Vivier, E., "Signaling pathways engaged by NK cell receptors: double concerto for activating receptors, inhibitory receptors and NK cells", 2000, *Seminars in Immunology*, 12, 139-147.
4. [5703] Sivori, S., Vitale, M., Morelli, L., Sanseverino, L., Augugliaro, R., Bottino, C., Moretta, L., Moretta, A., "p46, a novel natural killer cell-specific surface molecule that mediated cell activation", 1997, *J. Exp. Med.*, 7, 186, 1129-1136.
5. [5706] Pessino, A., Sivori, S., Bottino, C., Malaspina, A., Morelli, L., Moretta, L., Biassoni, R., Moretta, A., "Molecular cloning of NKp46: a novel member of the immunoglobulin superfamily involved in triggering of natural cytotoxicity", 1998, *J. Exp. Med.*, 5, 188, 953-960.
6. [5720] Vitale, M., Bottino, C., Sivori, S., Sanseverino, L., Castriconi, R., Marcenaro, E., Augugliaro, R., Moretta, L., Moretta, A., "NKp44, a novel triggering surface molecule specifically expressed by activated natural killer cells, is involved in non-major histocompatibility complex-restricted tumor cell lysis", 1998, *J. Exp. Med.*, 12, 187, 2065-2072.
7. [5705] Sivori, S., Pende, D., Bottino, C., Marcenaro, E., Pessino, A., Biassoni, R., Moretta, L., Moretta, A., "NKp46 is the major triggering receptor involved in the natural cytotoxicity of fresh or cultured human NK cells. Correlation between surface density of NKp46 and natural cytotoxicity against autologous, allogeneic or xenogeneic target cells", 1999, *Eur. J. Immunol.*, 29, 1656-1666.
8. [5697] Spaggiari, G.M., Carosio, R., Pende, D., Marcenaro, S., Rivera, P., Zocchi, M.R., Moretta, L., Poggi, A., "NK cell-mediated lysis of autologous antigen-presenting cells is triggered by the engagement of the phosphatidylinositol 3-kinase upon ligation of the natural cytotoxicity receptors NKp30 and NKp46", 2001, *Eur. J. Immunol.*, 31, 1656-1665.
9. [5704] Mandelboim, O., Lieberman, N., Lev, M., Paul, L., Arnon, T.I., Bushkin, Y., Davis, D.M., Strominger, J.L., Yewdell, J.W., Porgador, A., "Recognition of haemagglutinins on virus-infected cells by NKp46 activates lysis by human NK cells", 2001, *Nature*, 409, 1055-1060.
10. [5709] Vankayalapati, R., Wizel, B., Weis, S.E., Safi, H., Lakey, D.L., Mandelboim, O., Samten, B., Porgador, A., Barnes, P.F., "The NKp46 receptor contributes to NK cell lysis of mononuclear phagocytes infected with an intracellular bacterium", 2002, *J. Immunol.*, 168, 3451-3457.
11. [5707] Hashimoto, S., Toba, K., Tsuschiyama, J., Abe, T., Ano, T., Momoi, A., Okazuka, K., Kanazawa, N., Takahashi, M., Aizawa, Y., "CD56⁺, NKp46⁺ cell line (MZ93 expressing

T-cell and myeloid antigens", 2002, *Leuk. Res.*, 26, 289-295.

12. [5696] Maki, G., Klingemann, H-G., Martinson, J.A., Tam, Y.K., "Factors regulating the cytotoxic activity of the human natural killer cell line, NK-92", 2001, *J. Hematother. Stem Cell Res.*, 10, 369-383.
13. [5695] de Maria, A., Biassoni, R., Fogli, M., Rizzi, M., Cantoni, C., Costa, P., Conte, R., Mavilio, D., Ensoli, B., Cafaro, A., Moretta, A., Moretta, L., "Identification, molecular cloning and functional characterization of NKp46 and NKp30 natural cytotoxicity receptors in Macaca fascicularis NK cells", 2001, *Eur. J. Immunol.*, 31, 3546-3556.
14. [5694] Pende, D., Parolini, S., Pessino, A., Sivori, S., Augugliaro, R., Morelli, L., Marcenaro, E., Accame, L., Malaspina, A., Biassoni, R., Bottino, C., Moretta, L., Moretta, A., "Identification and molecular characterization of NKp30, a novel triggering receptor involved in natural cytotoxicity mediated by human natural killer cells", 1999, *J. Exp. Med.*, 10, 190, 1505-1516.
15. [5721] Costello, R.T., Sivori, S., Marcenaro, E., Lafage-Pochitaloff, M., Mozziconacci, M.J., Reviron, D., Gastaut, J.A., Pende, D., Olive, D., Moretta, A., "Defective expression and function of natural killer cell-triggering receptors in patients with acute myeloid leukemia", 2002, *Blood*, 10, 99, 3661-3667.

PRODUCT AVAILABILITY

IOTest NKp46-PE Conjugated Antibodies
PN IM3711 – 50 tests – 20 µL / test

PE is licensed under patent 4,520,110

For additional information in the USA, call 800-526-7694.

Outside the USA, contact your local Beckman Coulter representative.

TRADEMARKS

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