IOTest CD335 (NKp46)-PC7

PN B38703 – 0.5 mL – Liquid – Clone BAB281

Analyte Specific Reagent.
Analytical and performance characteristics are not established.

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 Fcγ RIII (CD16). Via multiple receptors, NK cells can select (with Natural Killer Receptors: NKRs) and lyse (with NKG2D, and Natural Cytotoxicity Receptors: NCRs) a variety of target cells (2). Unlike NKRs, NCRs are strictly restricted to NK cells. To date, and beside many existing coreceptors, 3 NCRs have been identified: CD335 (NKp46), CD336 (NKp44) and CD337 (NKp30). Their common features are (1):

- a direct involvement in target cell recognition and killing;
- the delivery of activating signals by association with ITAM-containing molecules (KARAP / DAP12, or CD3ζ, or FcεRIγ);
- 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 cytolysis.

The discovery of CD335 also known as NKp46 resulted from the selection of monoclonal antibodies that triggered NK-mediated target cell lysis (3). BAB281 was selected on these criteria, and helped identify CD335, a 46 kDa transmembrane glycoprotein, strictly expressed by all NK cells, including the infrequent CD3–CD56+CD16– subset (3).

The molecular cloning of CD335 (4), and biochemical studies (5), revealed that CD335 is associated with the ITAM-containing CD3ζ, although the CD335 expression on the cell surface does not require that of CD3ζ. The surface density of CD335 is linked to the cytolytic activity of NK cells (6). CD335, as CD337, is involved in the interaction of NK cells with autologous antigen-presenting cells (7).

Beyond its role in triggering natural cytotoxicity, the cellular distribution of CD335 is noteworthy: on freshly isolated PBMCs, CD335 is strictly restricted to CD3–CD56+ cells, including CD56bright/CD16–, in addition, CD335 is not expressed by the rare CD16+ CD56+ T cells. The monoclonal antibody BAB281 was first studied on human NK cells (3) and exhibits the following properties:

- BAB281 reacts with all and only mature NK cells, also in Macaques (8);
- BAB281 binding to CD335 induces a strong increase of cytolytic activity in redirected killing assays, also in Macaque (8);
- this triggering was also observed on $CD16^-$ NK cells, which did not respond to CD16 antibodies;
- the CD335-induced triggering was abolished by the simultaneous cross-linking of KIRs and CD94 / NKG2A (3);
- BAB281 binding to CD335 induces mobilization of intracellular Calcium;
- BAB281 masking of CD335 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 (6).

The BAB281 monoclonal antibody has been assigned to the CD335 cluster of differentiation during the 8th HLDA Workshop on Human Leucocyte Differentiation Antigens, held in Adelaide, Australia, in 2004 (9)

REAGENT

IOTest CD335 (NKp46)-PC7 Conjugated Antibody
PN B38703 - 0.5 mL - Liquid

Clone	BAB281
Isotype	IgG1 kappa, Mouse
Immunogen	Human NK clone
Hybridoma	P3U1 x balb/c
Source	Ascites fluid or supernatant of in vitro cultured hybridoma cells.
Purification	Affinity chromatography
Conjugation	R Phycoerythrin-Cyanine 7 (PC7)
Molar Ratio	PC7 / Ig : 0.5 - 1.5
Fluorescence	Excites at 488 nm Emits at 770 nm

REAGENT CONTENTS

This antibody is provided in phosphate-buffered saline, containing 0.1% sodium azide and 2 mg/mL bovine serum albumin.
Concentration: See lot specific Certificate of Analysis at www.beckmancoulter.com.

STATEMENTS OF WARNING

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 considered potentially infectious 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.
7. Use good laboratory practices when handling this reagent.
8. Any change in the physical appearance of the reagents may indicate deterioration and the reagent should not be used.

STORAGE AND HANDLING CONDITIONS AND STABILITY

This reagent is stable up to the expiration date when stored at 2 – 8°C. Do not freeze.
No reconstitution is necessary. This monoclonal antibody may be used directly from the vial. Bring reagent to 18 – 25°C prior to use.

PRECAUTIONS

Due to the tandem structure of the fluorochrome, PC7 also emits light at 575 nm. This secondary emission peak varies from lot-to-lot of PC7. Therefore, for multi-color analysis, the compensation matrix should be carefully checked when changing the lot of a PC7-conjugate.

SELECTED RESEARCH REFERENCES

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8. 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.
9. Florian S., Sonneck K., Czerny M., Hennersdorf F., Hauswirth A. W., Boehring H.-J., Valent P., “Detection of novel leukocyte differentiation antigens on basophils and mast cells by HLDA8 antibodies”, 2006, Allergy, 61, 1054-1062.

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