

Monoclonal Antibody IOTest[®] CD16-APC-Alexa Fluor[®] 750

PN A66330 - 50 tests - Liquid - 10 µL/test* - Clone 3G8

Analyte Specific Reagent.

Analytical and performance characteristics are not established.

SPECIFICITY

The CD16 antigen is the low-affinity receptor for IgG (Fc_γRIII) that binds immune complexes, but not monomeric IgG. The CD16 antigen exists in two different forms encoded by two different genes: Fc_γRIIIA (or III-2) and Fc_γRIIIB (or III-1). The genetic heterogeneity of CD16 generates alternative membrane-anchored molecules. One is a transmembrane form (Fc_γRIIIA, 50 – 65 kDa) expressed on NK cells, monocytes and macrophages. The other is a glycosylphosphatidylinositol (GPI)-anchored form (Fc_γRIIIB, 48 kDa) only expressed on neutrophils (1, 2).

It has been shown that the CD16 antigen can be non covalently associated within the membrane of NK cells, to the 16 kDa CD3_ζ chain (3), or to the dimeric Fc_γ chain (4).

The 3G8 monoclonal antibody (mAb) binds to the Fc_γRIIIA as well as the Fc_γRIIIB (strongly). It was shown to block almost completely the binding of IgG dimers to Fc_γRIIIB (5). Experiments where amino acid mutations were done on the Fc_γRIIIB molecule, showed that the 3G8 mAb is affected by Lys162 and Val164 substitutions in the FG loop of the membrane-proximal Ig-like domain of the molecule (6).

The 3G8 mAb was assigned to CD16 during the fifth HLDA workshop on Human Leucocyte Differentiation Antigens, held in Boston, USA, in 1993 (7).

REAGENT

IOTest CD16-APC-Alexa Fluor 750
Conjugated Antibody
PN A66330 - 50 tests - Liquid - 10 µL/test*

Clone	3G8
Isotype	IgG1 kappa, Mouse
Immunogen	Human Neutrophils
Hybridoma	SP2/0 x Balb/c
Source	Ascites fluid
Purification	Ion exchange or affinity chromatography
Conjugation	Allophycocyanin - Alexa Fluor 750 (APC-Alexa Fluor 750)
Molar Ratio	APC-Alexa Fluor 750 / Ig : 0.5 - 1.5
Fluorescence	Excites at 633 nm Emits at 783 nm

REAGENT CONTENTS

This antibody is provided in phosphate-buffered saline, containing 0.1% sodium azide and 2 mg/mL bovine serum albumin.

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 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.
7. Use good laboratory practices when handling this reagent.

STORAGE CONDITIONS AND STABILITY

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

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.

PRECAUTIONS

Due to the tandem structure of the fluorochrome, APC-Alexa Fluor 750 also emits light at 660 nm. This secondary emission peak varies from lot-to-lot of APC-Alexa Fluor 750. Therefore, for multi-color analysis, the compensation matrix should be carefully checked when changing the lot of a APC-Alexa Fluor 750-conjugate.

SELECTED RESEARCH REFERENCES

1. Ravetch, J.V., Perussia, B., "Alternative membrane forms of Fc_γRIII (CD16) on human natural killer cells and neutrophils", 1989, J. Exp. Med., 170, 481-497.
2. Huizinga, T.W.J., Roos, D., Von dem Borne, A.E.G. Kr., "Neutrophil Fc_γ receptors: A two-way bridge in the immune system", 1990, Blood, 75, 1211-1214.

3. Lanier, L.L., Yu, G., Phillips, J.H., "Co-association of CD3d_ζ with a receptor (CD16) for IgG Fc on human natural killer cells", 1989, Nature, 342, 803-805.
4. Hibbs, M., L., Selvaraj, P., Carpen, O., Springer, T.A., Kuster, H., Jouvin, M.-H., Kinet, J.-P., "Mechanisms for regulating expression of membrane isoforms of Fc_γRIII (CD16)", 1989, Science, 246, 1608-1611.
5. Tamm, A., Schmidt, R.E., "The binding epitopes of human CD16 (Fc_γRIII) monoclonal antibodies: Implication for ligand binding", 1996, J. Immunol., 157, 1576-1581.
6. Tamm, A., Bassmann, Schmidt, R.E., "Natural killer cell structural studies: Localization of the epitopes of human CD16 (Fc_γRIII) monoclonal antibodies on the molecular model of CD16", 1997, Leucocyte Typing VI, White Cell Differentiation Antigens. Kishimoto, T., et al, Eds., Garland Publishing, Inc., 324-326.
7. Ritz, J., Trinchieri, G., Lanier, L.L., "NK-cell antigens: section report", 1995, Leucocyte Typing V, White Cell Differentiation Antigens. Schlossman, S.F., et al., Eds., Oxford University Press, 1367-1372.

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(*): 10 µL is the quantity of product sufficient to stain
5 x 10⁵ cells in a standard immunofluorescence assay

