

## Analyte Specific Reagent.

Analytical and performance characteristics are not established.

### SPECIFICITY

The CD16 antigen is the low-affinity receptor for IgG (Fc $\gamma$ RIII) that binds immune complexes, but not monomeric IgG. The CD16 antigen exists in two different forms encoded by two different genes: Fc $\gamma$ RIIIA (or III-2) and Fc $\gamma$ RIIIB (or III-1). The genetic heterogeneity of CD16 generates alternative membrane-anchored molecules. One is a transmembrane form (Fc $\gamma$ RIIIA, 50 – 65 kDa) expressed on NK cells, monocytes and macrophages. The other is a glycosylphosphatidylinositol (GPI)-anchored form (Fc $\gamma$ RIIIB, 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 $\zeta$  chain (3), or to the dimeric Fc $\gamma$ R chain (4). The 3G8 monoclonal antibody (mAb) binds to Fc $\gamma$ RIIIA as well as to Fc $\gamma$ RIIIB (strongly). It was shown to block almost completely the binding of IgG dimers to Fc $\gamma$ RIIIB (5). Experiments where amino acid mutations were done on the Fc $\gamma$ RIIIB 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 has been assigned to the CD16 cluster of differentiation at the fifth International Workshop on Human Leucocyte Differentiation Antigens held in Boston, USA, in 1993 (7)

### REAGENT

IOTest CD16-APC-Alexa Fluor 700  
Conjugated Antibody  
PN B20023 - 0.5 mL - Liquid

<b>Clone</b>	3G8
<b>Isotype</b>	IgG1, Mouse
<b>Immunogen</b>	Human neutrophils
<b>Hybridoma</b>	SP2/0 x balb/c
<b>Source</b>	Ascites fluid or supernatant of in vitro cultured hybridoma cells.
<b>Purification</b>	Affinity chromatography
<b>Conjugation</b>	Allophycocyanin-Alexa Fluor 700 (APC-Alexa Fluor 700)
<b>Molar Ratio</b>	APC-Alexa Fluor 700 / Ig : 0.5 - 1.5
<b>Fluorescence</b>	Excites at 633/638 nm Emits at 720 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](http://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 with 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, APC-Alexa Fluor 700 also emits light at 660 nm. This secondary emission peak varies from lot-to-lot of APC-Alexa Fluor 700. Therefore, for multi-color analysis, the compensation matrix should be carefully checked when changing the lot of a APC-Alexa Fluor 700-conjugate.

Weak non-specific binding on a lymphocyte subpopulation may occur on some donors with APC-Alexa Fluor 700 conjugates.

### SELECTED RESEARCH REFERENCES

1. Ravetch, J.V., Perussia, B., "Alternative membrane forms of Fc $\gamma$ RIII (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 $\gamma$  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 $\zeta$  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 $\gamma$ RIII (CD16)", 1989, Science, 246, 1608-1611.
5. Tamm, A., Schmidt, R.E., "The binding epitopes of human CD16 (Fc $\gamma$ RIII) 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 $\gamma$ RIII) 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.

### TRADEMARKS

Beckman Coulter, the stylized logo, and IOTest are trademarks of Beckman Coulter, Inc., and registered with the USPTO.

### MANUFACTURED BY :

IMMUNOTECH SAS  
a Beckman Coulter Company  
130, avenue de Lattre de Tassigny  
B.P. 177 - 13276 Marseille Cedex 9  
France

For additional information, or if damaged product is received, call Beckman Coulter Customer Service at 800-526-7694 (USA or Canada) or contact your local Beckman Coulter Representative.

[www.beckmancoulter.com](http://www.beckmancoulter.com)

Printed in France.  
Made in France.

©2012 Beckman Coulter, Inc.