

## Analyte Specific Reagent.

Analytical and performance characteristics are not established.

### SPECIFICITY

The CD64 molecule is a single chain, heavily N-glycosylated type I transmembrane protein, with a molecular weight of 72 kDa. After removal of the N-linked carbohydrates, a core protein of 55 kDa is found (1).

CD64 is also known as the high-affinity receptor for IgG (FcγRI). It is one, with CD32 (FcγRII) and CD16 (FcγRIII), of three distinct receptors for IgG found on human leukocytes. CD64 has structural similarity with CD32, CD16, as well as with FcεRIα (2). The CD64 molecule binds polymeric or aggregated-IgG as the other Fcγ receptors do. However, it is the only receptor able to bind monomeric IgG with an subclass-specific affinity (i.e. decreasing affinity for IgG subclasses: IgG1 > IgG3 >> IgG4 >>> IgG2) (1, 2).

In contrast with CD32 and CD16, which include two immunoglobulin-like (Ig-like) extracellular domains, CD64 presents three Ig-like extracellular domains.

The CD64 molecule is constitutively expressed on monocytes, macrophages, and a subset of dendritic cells (3, 4). The expression of CD64 on polymorphonuclear neutrophils is weak but can be upregulated by interferon-γ (IFN-γ) or granulocyte colony-stimulating factor (G-CSF).

CD64 is involved in antibody-dependent cytotoxicity, clearance of immune complexes, and phagocytosis of IgG opsonized targets. The association of CD64 with the Fc receptor γ-chain homodimer is required for its signal transduction activity (γ-chain is also a subunit of FcεRI, FcγRIIIA (CD16), and FcαR (CD89) (5, 6).

The 22 monoclonal antibody has been assigned to the CD64 cluster of differentiation at the 4th International Workshop on Human Leukocyte Differentiation Antigens in Vienna, Austria, in 1989 (WS Code: 96, Section: M) (7).

### REAGENT

IOTest CD64-PC5 Conjugated Antibody  
PN IM3606U – 1 mL Liquid – 10 µL / test\*.

**Clone** 22

**Isotype** IgG1, mouse

**Immunogen** Human monocytes

**Hybridoma** P3/NS/1-Ag4-1 x Balb/c

**Source** Ascites fluid

**Purification** Ion exchange or affinity chromatography

**Conjugation** PC5: The Ig is conjugated to a tandem dye constituted of R-phycoerythrin covalently linked to Cy5 at 0.5 – 1.5 mole of PC5 per mole of Ig.

**Fluorescence** PC5 (Deep Red)  
Excites at 486–580 nm  
Emits at 660–680 nm

### REAGENT CONTENTS

This reagent is provided in phosphate-buffered saline, with 0.1% sodium azide (NaN<sub>3</sub>) as preservative, and 2.0 mg / mL bovine serum albumin (BSA).

### 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. Do not use antibody beyond the expiration date on the label.
3. Samples and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
4. Never pipet by mouth and avoid contact of samples with skin and mucous membranes
5. Minimize exposure of reagent to 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. Minimize exposure to light.

### EVIDENCE OF DETERIORATION

Any change in the physical appearance of this PC5-labeled reagent (clear colorless to pinkish liquid) or any major variation in values obtained for control samples may indicate deterioration and the reagent should not be used.

### REAGENT PREPARATION

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

### SELECTED RESEARCH REFERENCES

1. Huizinga, T.W.J., Roos, D., von dem Borne, E.G.Kr., "Neutrophil Fcγ Receptor: a two-way bridge in the

immune system", 1990, *Blood*, 75, 1211-1214.

2. Ravetch, J.V., Kinetic, J.P., "Fc receptors", 1991, *Annu. Rev. Immunol.*, 9, 457-492.
3. Grage-Griebenow, E., Zawatzky, R., Kahlert, H., Brade, L., Flad, H.D., Ernst, M., "Identification of a novel dendritic cell-like subset of CD64<sup>+</sup>/CD16<sup>+</sup> blood monocytes", 2001, *Eur. J. Immunol.*, 31, 48-56
4. Grage-Griebenow, E., Flad, H.D., Ernst, M., "Heterogeneity of human peripheral blood monocyte subsets", 2001, *J. Leucocyte Biol.*, 69, 11-20
5. Ravetch, J.V., "Fc receptor", 1997, *Curr. Opin. Immunol.*, 9, 121-125.
6. Masuda, M., "Association of all three types of FcγR (CD64, CD32, and CD16) with a γ-chain homodimer in cultured human monocytes", 1993, *J. Immunol.*, 151, 7188.
7. Majdic, O., "Cluster report: CD64", 1989, *Leucocyte Typing IV, White Cell Differentiation Antigens*. W. Knapp, et al., Eds., Oxford University Press, 835-836.

### PRODUCT AVAILABILITY

IOTest CD64-PC5 Conjugated Antibodies  
PN IM3606U – 1 mL Liquid – 10 µL / test\*.

PC5 is licensed under patents 4,542,104 and 4,520,110.  
Cy5 is licensed under patents 4,981,977 and 5,268,486.

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

Outside the USA, contact your local Beckman Coulter representative.

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(\*) : 10 µL is the quantity of product sufficient to stain

5 x 10<sup>5</sup> cells in a standard immunofluorescence assay