

PN IM3601**100 tests
20 µL/test****CD64-PE****(22)**
IOTest®
 Conjugated Antibodies

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

SPECIFICITY

The CD64 is a single chain, type I transmembrane molecule with a molecular weight of 72 kDa. The core protein with a molecular weight close to 55 kDa is heavily N-glycosylated (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). CD64 molecule binds polymeric or aggregated-IgG. However it is the only receptor able to bind monomeric IgG with an affinity subclass-specific (i. e. decreasing affinity for IgG subclass: IgG1 > IgG3 >> IgG4 >> IgG2) (1, 2).

In contrast with CD32 and CD16 constituted by two immunoglobulins-like (Ig-like) extracellular domains, CD64 molecule presents three Ig-like extracellular domains.

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-dependant 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

Clone	22
Isotype	IgG1
Immunogen	Human monocytes
Hybridoma	F3/NS1-AG4-1 X balb/c
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 studies of CD64 expressing cells.
 Flow cytometry studies of neutrophils activation.
 Flow cytometry characterization of dendritic cells (3,4)
 Identification and characterization of myelogenous lineage differentiation (i.e. CD64^{pos}) in hematopoietic acute malignancies (8).

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

Each 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

This reagent is designed for flow cytometry.

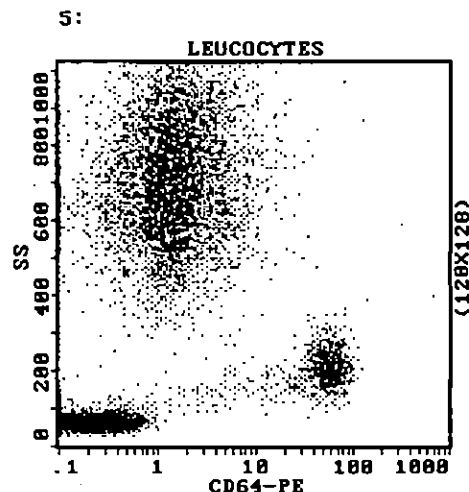
Assay volume: 20 µL per 5 x 10⁵ cells in one test, or per 100 µL whole blood.

A wash is required to yield optimal results.

EXAMPLE DATA

The histograms below are biparametric representations (Side Scatter versus Fluorescence Intensity) of a human whole blood. Staining is with CD64-PE monoclonal antibody (PN IM3601) on leukocytes. Erythrocytes lysis is with the "Whole Blood Lysing Reagent Kit" (PN 6602764 or 6603152). Isotypic control (PN IM0670) labeling is not shown.

Figure 1: Acquisition is with a COULTER® EPICS® XL™ flow cytometer. Analysis is with the XL System II™ software.



3601EX160701 20/07/01...AC-01-0532


COULTER

PARTNERS IN CELL ANALYSIS

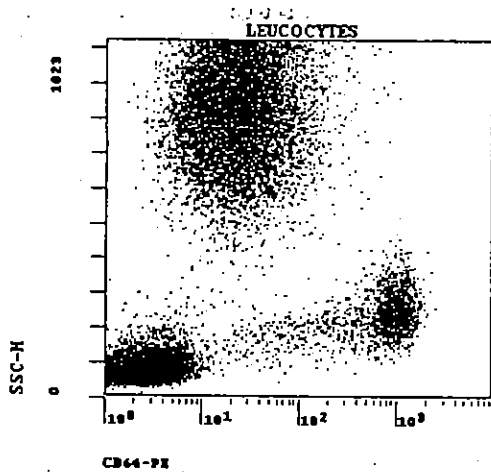

IMMUNOTECH
 A COULTER COMPANY

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Figure 2: Acquisition is with a BD Biosciences FACScan™ flow cytometer equipped with LYSYS II™ software. Analysis is with EXPO™ Cytometer software (PN 6605434)



SELECTED RESEARCH REFERENCES

- [4913] Huizinga, T.W.J., Roos, D., von dem Borne, E.G.Kr. "Neutrophil Fc-γ Receptor: a two-way bridge in the immune system" 1990
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- [5625] Grage-Griebenow, E., Zawatzky, R., Kahlert, H., Brade, L., Flad, H.D., Ernst, M., "Identification of a novel dendritic cell-like subset of CD64+/CD16+ blood monocytes", 2001, Eur.J.Immunol, 31, 48-56.
- [5626] Grage-Griebenow, E., Flad, H.D., Ernst, M., "Heterogeneity of human peripheral blood monocyte subsets", 2001, J.Leucocyte Biol, 69, 11-20.
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- [2122] Masuda, M., "Association of all three types of γR (CD64, CD32, and CD16) with a γ-chain homodimer in cultured human monocytes" 1993, Immunol., 12, 151, 71-88.
- [3213] Majdic, O. "Cluster report : CD64" 1989
- [3470] Stewart, C.C., Behm, F.G., Carey, J.L., Combleet, J., Duque, R.E., Hudnall, S.D., Hurtubise, P.E., Loken, M., Tubbs, R.R., Wormsley, S. "U.S. Canadian consensus recommendations on the immunophenotypic analysis of hematologic neoplasia by flow cytometry : selection of antibody combinations" 1997.

3801EX160701 20/07/01...AC-01-0532

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