# IOTest® CD14-PC7

## PN A22331 – 100 tests – 10 µL/test – Clone RMO52

**For Research Use Only. Not for use in diagnostic procedures.**

### SPECIFICITY

The CD14 molecule is a protein with a molecular weight of 53 – 55 kDa anchored in the membrane by means of a glycosyl-phosphatidylinositol group (GPI) (1).
The CD14 antigen is strongly expressed on monocytes and macrophages and moderately so on peripheral blood polynuclear neutrophils; it is also present on pleural phagocytes and dendritic reticular cells. The RMO52 monoclonal antibody (mAb) does not react with B and T lymphocytes or peripheral blood granulocytes. CD14 is found on cells of the myelo-monocytic line and is only very weakly expressed by B-lymphocytes. It is absent from T lymphocytes as well as from NK cells, erythrocytes and platelets (2 - 5).
Studies have shown that CD14 can function as a receptor for lipopolysaccharide (LPS). LPS-CD14 binding is markedly enhanced by the presence of a plasma protein, LPS-binding protein (LBP).
The RMO52 mAb was assigned to CD14 during the 6th HLDA Workshop on Human Leucocyte Differentiation Antigens, held in Kobe, Japan, in 1996 (WS Code: MA62, Section M) (6).

### REAGENT

IOTest CD14-PC7 conjugated Antibody
PN A22331 – 100 tests – 10 µL/test

| | |
|---|---|
| **Clone** | RMO52 |
| **Isotype** | IgG2a |
| **Immunogen** | Isolated human monocytes |
| **Hybridoma** | SP2/0 x Balb/c spleen cells |
| **Source** | Ascites fluid |
| **Purification** | Ion exchange or affinity chromatography |
| **Conjugation** | Phycoerythrin Cyanin 7 (PC7) The IgG is conjugated to a tandem dye constituted of R-phycoerythrin covalently linked to cyanine 7 (indotri-carbocyanine) at 0.5 — 1.5 mole of PC7 per mole of Ig. |

Excitation wavelength: 488 nm
Emission wavelength range: 750 — 810 nm
Main emission color: Far-red

**Buffer** 2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide.

### APPLICATION

Flow cytometry.

### 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.
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.

### 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

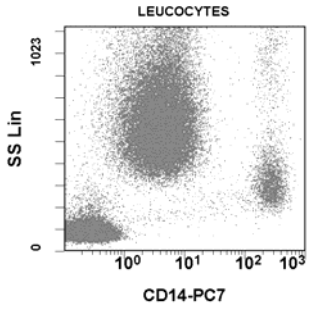
Due to the tandem structure of the fluorochrome, PC7 also emits light at 575 nm. This secondary emission peak varies from lot-to-lot of PC7. Therefore, for multi-color analysis, the compensation matrix should be carefully checked when changing the lot of a PC7-conjugate

### PROCEDURE

This reagent is designed for flow cytometry.
Assay volume: 10 µL / 5 x $10^5$ cells (or 100 µL whole blood), per test.
A wash is required to yield optimal results.

### EXAMPLE DATA

The graph below is a biparametric representation (Side Scatter versus Fluorescence Intensity) of lyzed normal whole blood sample.
Staining is with CD14-PC7 monoclonal antibody (PN A22331). Gate is on all leucocytes, emphasizing on the bright staining of monocytes and the dim staining of neutrophils.
Acquisition and analysis are performed with a CYTOMICS FC 500 flow cytometer equipped with CXP Analysis Software.




### SELECTED RESEARCH REFERENCES


1. Todd III, R.F., Nadler, L.M., Schlossman, S.F., "Antigens on human monocytes identified by monoclonal antibodies", 1981, J. Immunol., 4, 126, 1435-1442.
2. Todd, R.F., van Agthoven, A., Schlossmann, S.F., Terhorst, C., "Structural analysis of differentiation antigens Mo1 and Mo2 on human monocytes", 1982, Hybridoma, 3, 1, 329- 337.
3. Lauener, R.P., Geha, R.S., Verceli, D., "Engagement of the monocyte surface antigen CD14 induces lymphocyte function-associated antigen/intercellular adhesion molecule-1-dependant homotypic adhesion", 1990, J. Immunol., 5, 145, 1390-1394.
4. Peters, J.H., Ruppert, J., Gieseler, R.K.H., Najar, H.M., Xu, H., "Differentiation of human monocytes into CD14 negative accessory cells: do dendritic cells derive from the monocytic lineage?", 1991, Pathobiology, 59, 122-126.
5. Ziegler-Heitbrock, H.W.L., Ulevitch, R.J., "CD14: Cell surface receptor and differentiation markers", 1993, Immunol. Today, 3, 14, 121-125.
6. Goyert, S.M., Cohen, L., Gangloff, S.C., Ashmun, R., Haeffner-Cavaillon, N., "CD14 Workshop panel report", 1997, Leucocyte Typing VI, White Cell Differentiation Antigens. Kishimoto, T., et al, Eds., Garland Publishing, Inc., 963-965.


### PRODUCT AVAILABILITY

IOTest CD14-PC7 conjugated Antibody
PN A22331 – 100 tests – 10 µL/test

PE is licensed under patent 4,520,110



I-A22331EX 2007-01-09

BECKMAN COULTER

For additional information in the USA, call 800-526-7694.
Outside the USA, contact your local Beckman Coulter representative.
***www.beckmancoulter.com***





Manufactured by:
Immunotech SAS, a Beckman Coulter Company
130, avenue de Lattre de Tassigny, B.P. 177
13276 Marseille Cedex 9, France