MONOCLONAL ANTIBODY CD14

Cat. No.	Form	Quantity	Presentation
0643	Purified	0.2 mg	Freeze-dried
0644	Purified	100 tests	Liquid 2 mL
~~0645~~	~~FITC~~	~~100 tests~~	~~Liquid 2 mL~~
~~0650~~	~~PE~~	~~100 tests~~	~~Liquid 2 mL~~
1852	PE-Cy5	100 tests	Liquid 2 mL
2580	APC	100 tests	Liquid 1 mL

Warning ***APC-conjugated forms of the IOTest® line of reagents are to be used at 10 µL / test instead of 20 µL / test.***

Clone RMO52

Isotype IgG2a κ (mouse)

Immunogen Isolated human monocytes

Hybridoma SP2/0 x Balb/c spleen cells

Specificity CD14 is a 55 kDa glycoprotein expressed on peripheral blood monocytes, on macrophages, and polymorphonuclear leukocytes. It is also present on pleural phogocytic cells and on reticular dendritic cells.

Studies have shown that CD14 can function as a receptor for lipopolysaccharide (LPS) .LPS-CD14 binding is enhanced markedly by the presence of a plasma protein, LPS-binding protein (LBP).

RMO52 does not react with B and T lymphocytes or peripheral blood granulocytes.

Applications Studies of monocytes, and macrophages from blood, bone marrow, fluid and tissue.

Studies have shown that anti-CD14 antibodies can be useful in the identification of monocytic and myelomonocytic leukemias.

Buffer Freeze-dried forms: 1 mg/ml bovine serum albumin in phosphate buffered saline.

Liquid forms: 2 mg/ml bovine serum albumin in phosphate buffered saline containing 0.1% sodium azide.

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Conjugation

FITC: Fluorescein isothiocyanate (FITC) is conjugated at 6-10 moles of FITC per mole of IgG.
Excitation wavelength: 488 nm
Maximum emission wavelength: 525 nm
Main emission color: Green

PE: R-phycoerythrin (PE) is conjugated at 0.7-1 mole of PE per mole of IgG.
Excitation wavelength: 488 nm
Maximum emission wavelength: 575 nm
Main emission color: Orange-red

PE-Cy5: The IgG is conjugated to a tandem dye constituted of R-phycoerythrin covalently linked to cyanin 5.1 at 0.7-1 mole of PE-Cy5 per mole of IgG.
Excitation wavelength: 488 nm
Maximum emission wavelength: 670 nm
Main emission color: Deep-red

APC: Allophycocyanin (APC) is conjugated at 0.7-1 mole of APC per mole of IgG.
Excitation wavelength: 633-635 nm
Maximum emission wavelength: 660 nm
Main emission color: Deep-red

Limitation: APC conjugates are recommended for use only on flow cytometers equipped with an exciting source of 633 nm (He-Ne laser) or 635 nm (Red diode laser).

Reconstitution and Storage

The freeze-dried form may be stored at 2-8°C until the expiration date. Reconstitute with 1 ml of distilled water. No preservative has been added. The reconstituted form may be stored at -20°C until the expiration date. Aliquotting is suggested to avoid multiple freeze-thaw cycles. The addition of sodium azide at 0.1% (w/v) is recommended for storage of the reconstituted form for up to one month at 2-8°C.

The purified liquid form should be stored at 2 - 8°C.

The conjugated forms should not be frozen and should be stored in the dark at 2 - 8°C until the expiration date stated on the vial label.

Recommended Procedures

Flow cytometry:
Liquid form: 20 µL /5 x 10^5cells/test or 100 µL whole blood
Freeze-dried form: 2 µg /5 x 10^5/test

Limitation : PE-Cy5 conjugates are recommended for use only on flow cytometers equiped with a 675 nm band pass filter in front of the third fluorescence detector

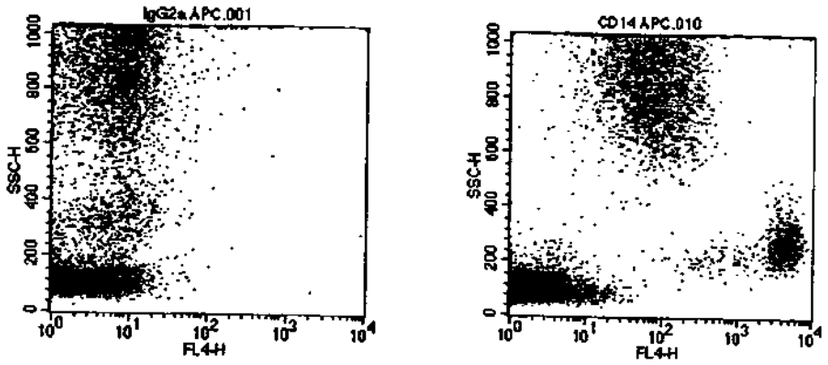
Cat. No. 0643

APC-conjugated form: A specific calibration is applied to facilitate the blending of conjugated antibodies in multiparametric flow cytometry.
10 µL / 5 x 10^5 cells or 100 µL of whole blood.

Limitation: R-phycoerythrin (PE) is sensitive to light exposure. Consequently, PE- or PE-Cy5-conjugated antibodies are not suitable for fluorescence microscopy.

Example Data

The graphs below are double parameter representations (Side Scatter *versus* Fluorescence 4) of a lyzed whole blood sample from an healthy donor. Staining is with IgG2a-APC (left) and CD14-APC (right). Along the Y axis, lymphocytes are events with low side scatter values, monocytes show low to medium side scatter values and neutrophils show medium to high side scatter values.



Isotypic control: IgG2a-APC (Cat. No. 2476) Specific staining: CD14-APC (Cat. No. 2580)

Analysis is with a Becton Dickinson FACSCalibur™ flow cytometer equipped with CELLQuest™ Software.

IOTest is a registered trademark of Immunotech, a Coulter Company.

FACSCalibur and CELLQuest are trademarks of Becton Dickinson Immunocytometry Systems (BDIS).

References

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2) Todd III, R.F., Van Agthoven, A., Schlossman, S.F. & Terhorst, C., "Structural analysis of differentiation antigens Mo1 and Mo2 on human monocytes",1982, Hybridoma, **1**, 329-337.

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3) Lauener, R.P., Geha, R.S., Vercelli, D., "Engagement of the monocyte surface antigen CD14 induces lymphocyte function-associated antigen-1/intercellular adhesion molecule-1-dependant homotypic adhesion", 1990, J Immunol, **145**(5), 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?" 1990, Pathobiology, **59**, 122-126.

5) Ziegler-Heitbrock, H.W.L., Ulevitch, R.J., "CD14: Cell surface receptor and differentiation marker", 1993, Immunol Today, **14** (3), 121-125.