### MONOCLONAL ANTIBODY

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Form</th>
<th>Quantity</th>
<th>Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1301</td>
<td>Biotin</td>
<td>0.2 mg</td>
<td>Freeze-dried</td>
</tr>
<tr>
<td>1304</td>
<td>Purified</td>
<td>0.2 mg</td>
<td>Freeze-dried</td>
</tr>
<tr>
<td>1280</td>
<td>Purified</td>
<td>100 tests</td>
<td>Liquid 2 mL</td>
</tr>
<tr>
<td>1281</td>
<td>FITC</td>
<td>100 tests</td>
<td>Liquid 2 mL</td>
</tr>
<tr>
<td>1282</td>
<td>PE</td>
<td>100 tests</td>
<td>Liquid 2 mL</td>
</tr>
<tr>
<td>1371</td>
<td>PE-Cy5</td>
<td>100 tests</td>
<td>Liquid 2 mL</td>
</tr>
<tr>
<td>2467</td>
<td>APC</td>
<td>100 tests</td>
<td>Liquid 1 mL</td>
</tr>
</tbody>
</table>

**Warning**

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

**Clone**

UCHT1

**Isotype**

IgG1 k (mouse)

**Immunogen**

Peripheral blood lymphocytes

**Hybridoma**

NS1 x Balb/c spleen cells

**Specificity**

T lymphocytes constitute the majority of human peripheral blood lymphocytes (PBL) and are characterized by the expression of the CD3 antigen on the cell membrane (1). T lymphocytes are commonly divided into two subsets: "helper / inducer" lymphocytes expressing CD4 antigen and "suppressor / cytotoxic" lymphocytes expressing the CD8 molecule.

The CD3 antigen is a complex of 5 invariant polypeptide chains (γ, δ, ε, ζ and η) with molecular weights (Mₚ) of 25-28, 20, 20, 16 and 22 kDa, respectively. The ζ chain is frequently expressed as a disulfide linked homodimer in the CD3 complex.

CD3 is associated with the T cell receptor (TcR) on T lymphocytes, and is involved in the recognition of peptides bound to the major histocompatibility complex (MHC) during the immune response (2).

The CD3 antigen is expressed by mature T lymphocytes and by a subset of thymocytes (1, 3). In human PBL, approximately 67 to 76% of the lymphocytes are CD3+. The range of percentage is lower during childhood and may vary with aging (4).

The UCHT1 monoclonal antibody reacts with the ε chain of the CD3 complex (5).

The UCHT1 monoclonal antibody has been assigned to the CD3 cluster of differentiation at the 1st International Workshop on Human Leucocyte Differentiation Antigens in Paris (1982) (6).

**Applications**

Flow cytometry: Studies of CD3 expressing lymphocytes. This antibody is also useful for intracellular studies of the CD3ε chain.

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Buffer

Freeze-dried forms: 1 mg/mL of bovine serum albumin in phosphate-buffered saline

Liquid forms. 2 mg/mL of bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide.

Conjugation

FITC: Fluorescein isothiocyanate (FITC) is conjugated at 4-7 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 forms should be stored at 2-8°C until the expiration date stated on the vial label. Reconstitute with 1 ml of distilled water. No preservative has been added. The reconstituted forms should be stored at -20°C until the expiration date stated on the vial label. 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 forms for up to one month at 2-8°C.

The purified liquid form should be stored at 2-8°C until the expiration date stated on the vial label.

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:

Purified, FITC-, PE- and PE-Cy5-conjugated forms: 20 μL / 5 x 10^5 cells or 100 μL of whole blood.

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.
Results

The graphs below are double parameter representations (Side Scatter versus Fluorescence 4) of a lysed whole blood sample from an healthy donor. Staining is with IgG1-APC (left) or CD3-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, IgG1-APC (Cat. No. 2475)  Specific staining, CD3-APC (Cat. No. 2467)

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

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References


