

# IOTest<sup>®</sup> 3 Neg.Ctrl.-FITC / Neg.Ctrl.-PE / CD3-ECD

PN IM3670 – 25 tests – 20 µL / test

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

## REAGENT

IOTest 3 Conjugated Antibodies – Neg.Ctrl.-FITC / Neg.Ctrl.-PE / CD3-ECD  
PN IM3670 – Liquid – 20 µL / test

	CLONE 1	CLONE 2	CLONE 3
Specificity	N/A	N/A	CD3
Clone	679.1Mc7	679.1Mc7	UCHT1
Hybridoma	P3-X63-Ag.8.653 x Balb/c	P3-X63-Ag.8.653 x Balb/c	NS1 x Balb/c
Immunogen	Non-biological hapten	Non-biological hapten	Peripheral blood lymphocytes
Ig Chain	IgG1	IgG1	IgG1
Species	Mouse	Mouse	Mouse
Source	Ascites fluid	Ascites fluid	Ascites fluid
Purification	Ion exchange or affinity chromatography	Ion exchange or affinity chromatography	Ion exchange or affinity chromatography
Buffer	2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide.		
Conjugation	Fluorescein isothiocyanate (FITC)	PE (R-Phycoerythrin)	ECD <sup>™</sup> (Phycoerythrin-Texas-Red <sup>™</sup> -X)

N/A = not applicable

## SPECIFICITY

Hematopoietic cell differentiation is characterized by the expression of distinct membrane antigens at specific stages of the cellular maturation. These antigens expressed on the membrane are identified by specific monoclonal antibodies (mAb). Certain mAbs have irrelevant specificities: they induce non-specific immunolabeling on hematopoietic cells and platelets (1). The 679.1Mc7 mAb shares certain structural characteristics (i.e. isotypes and conjugated fluorochromes) with mAbs of interest (i.e. specific of hematopoietic cell surface antigens) but is devoid of any relevant specificities with regard to the studied cell population (1).

The CD3 antigen is a complex of 5 polypeptidic chains:  $\gamma$ ,  $\delta$ ,  $\epsilon$ ,  $\zeta$  and  $\eta$  associated with the T-cell receptor (TCR) complex (2). The CD3 antigen is expressed by mature T lymphocytes and by a subset of thymocytes (3).

The UCHT1 mAb reacts with the  $\epsilon$  chain of the CD3 complex (4). It has been assigned to the CD3 cluster of differentiation at the 1st International Human Leucocyte Differentiation Antigens Workshop in Paris, France, in 1982 (WS Code: 3, Section T) (5).

## CONJUGATION

- Fluorescein isothiocyanate (FITC) is conjugated at 2 – 5 moles of FITC per mole of Ig.  
Excitation wavelength: 488 nm  
Maximum emission wavelength: 525 nm  
Main emission color: Green
- 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

- R-phycoerythrin covalently linked to Texas Red (PE-TxR or ECD) is conjugated at 0.5 – 1.5 moles of ECD per mole of Ig.  
Excitation wavelength: 488 nm  
Maximum emission wavelength: 613 nm  
Main emission color: Red

## APPLICATION

This IOTest 3 reagent is a negative control for multiparametric flow cytometry analysis of leucocytes antigens expression using FITC/PE/ECD as fluorochromes and CD3-ECD for blasts gating (6).

The negative staining pattern related to each analyzed fluorescences (i.e. FITC, PE, and ECD) is designed to match the level of background fluorescence resulting from autofluorescence and nonspecific binding for each specific CD3<sup>pos</sup> gated populations (7).

## STATEMENT OF WARNINGS

- 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.
- 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.
- Never pipet by mouth and avoid contact of samples with skin and mucous membranes
- Do not use antibody beyond the expiration date on the label.
- Do not expose reagents to strong light during storage or incubation.

- Avoid microbial contamination of reagents or incorrect results might occur.

## 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.

## PROCEDURE

This reagent is designed for flow cytometry. Assay volume: 20 µL per 5 x 10<sup>5</sup> cells in one test, or per 100 µL whole blood or bone marrow.

A wash is required to yield optimal results. The use of IOTest 3 Lysing Solution (PN IM3514) and IOTest 3 Fixative Solution (PN IM3515) procedures are recommended to yield optimal results.

## EXAMPLE DATA

The 2 diagrams next page are biparametric representations of a normal peripheral whole blood specimen. Staining is with Neg.Ctrl.-FITC / Neg.Ctrl.-PE / CD3-ECD Conjugated Antibodies (PN IM3670). Lysis and fixation are with IOTest 3 Lysing Solution (PN IM3514) and IOTest 3 Fixative Solution (PN IM3515) respectively.

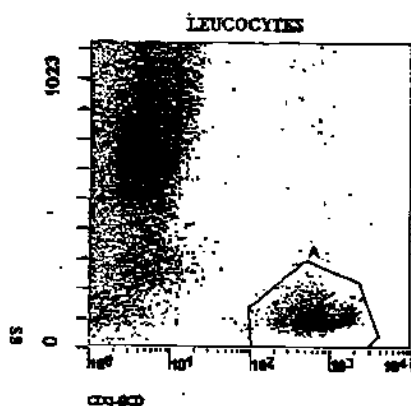
Acquisition is with a COULTER<sup>®</sup> EPICS<sup>®</sup> XL<sup>™</sup> flow cytometer equipped with System II<sup>™</sup> Software. Analysis is with the Beckman Coulter EXPO<sup>™</sup> 32 Software.

**Figure 1:**  
Biparametric representation (Fluorescence Intensity versus Side Scatter) in order to gate on positive events (i.e. CD3<sup>pos</sup>). All events acquired are shown in this representation.

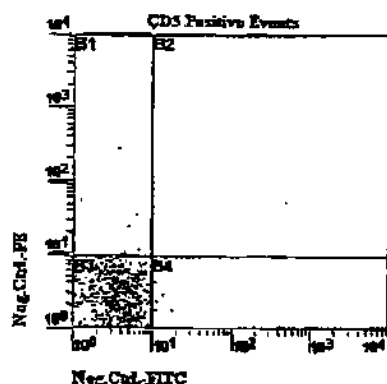
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**Figure 2:**  
Biparametric representation (Fluorescence Intensity versus Fluorescence Intensity) on CD3<sup>CD3-ECD</sup> gated events.



## SELECTED RESEARCH REFERENCES

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2. Thibault, G., Bardos, P., "Compared TCR and CD3 $\epsilon$  expression on  $\alpha\beta$  and  $\gamma\delta$  cells. Evidence for the association of two TCR heterodimers with three CD3 $\epsilon$  chains in the TCR / CD3 complex", 1995, *J. Immunol.*, 154, 3814-3820.
3. van Agthoven, A., Terhorst, C., Reinherz, E.L., Schlossman, S.F., "Characterization of T cell surface glycoproteins T1 and T3 present on all human peripheral T lymphocytes and functional mature T lymphocytes", 1981, *Eur. J. Immunol.*, 11, 18-21.
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5. Bernard, A., Brettlér, P., Georget, E., Lepage, V., Boumsell, L., "Joint report of the first International Workshop on Human Leucocyte Differentiation Antigens by the investigators of the participating laboratories", 1984, *Leucocyte Typing I*, Bernard, A. et al. Eds., Springer Verlag, 9-142.
6. Rothe, G., Schmitz, G., Adorf, D., Barlage, S., Gramatzki, M., Hoffkes, H.G., Janossy, G., Knüchel, R., Ludwig, W.D., Nebe, T., Nerl, C., Orfao, A., Serke, S., Sonnen, R., Tichelli, A., Wörmann, B., "Consensus protocol for the flow cytometric immunophenotyping

of hematopoietic malignancies", 1996, *Leukemia*, 10, 877-895.

7. Borowitz, M., Bauer, K.D., Duque, R.E., Horton, A.F., Marti, G., Muirhead, K.A., Peiper, S., Rickman, W., "Clinical applications of flow cytometry: quality assurance and immunophenotyping of lymphocytes; approved guideline", 1998, *NCCLS*, 21, 18.

## PRODUCT AVAILABILITY

IOtest 3 Neg.Ctrl.-FITC / Neg.Ctrl.-PE / CD3-ECD Conjugated Antibodies  
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## TRADEMARKS

IOtest<sup>®</sup> is a registered trademark of Immunotech S.A.

Texas Red<sup>®</sup> is a trademark of Molecular Probes, Inc.

PE is licensed under patent 4,520,110

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

Outside the USA, contact your local Beckman Coulter representative.

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