

PN IM3484**25 tests****20 µL/test****CD5-FITC****CD7-PE****CD3-ECD**
IO Test[®] 3
 Conjugated Antibodies

For Research Use Only. Not For Use In Diagnostic Procedures.
REAGENT
 IO Test 3 Conjugated Antibodies – CD5-FITC / CD7-PE / CD3-ECD
 PN IM3484 – 25 tests – 20 µL/test

	CLONE 1	CLONE 2	CLONE 3
Specificity	CD5	CD7	CD3
Clone	BL1a	8H8.1	UCHT1
Hybridoma	SP2/0-Ag14 x Balb/c	P3-X63-Ag.8.653 x Balb/c	NS1 x Balb/c
Immunogen	Human thoracic duct lymphocytes	Human thymocytes	Peripheral blood lymphocytes
Ig Chain	IgG2a	IgG2a	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™ (Phycoerythrin-Texas-Red®-X)

SPECIFICITY

The CD5 molecule is expressed on mature T lymphocytes, on most thymocytes and is also present on a subpopulation of B lymphocytes (1–3). It is not expressed on granulocytes, monocytes or platelets (3).

The BL1a monoclonal antibody (mAb) has been assigned to the CD5 cluster of differentiation during the 3rd International Workshop on Human Leucocyte Differentiation Antigens (HLDA) in Oxford, England, in 1986 (WS Code: 520, Section T) (1, 2).

The CD7 molecule is expressed at an early stage of T lineage ontogeny, during the extrathymic prothymocytic formation. CD7 expression persists throughout T-lymphocyte differentiation thus defining CD7 as a Pan-T marker (4–6). The CD7 glycoprotein is also expressed on thymocytes, on the majority of resting T-lymphocytes, and Natural Killer cells (NK), and on a subset of pre-B lymphocytes and B lymphocytes from foetal bone marrow (4, 7). CD7 expression is also detected on pluripotent hematopoietic stem cells (4). Mature B-lymphocytes, cells from erythroid, myeloid and megacaryocytic lineage do not express the CD7 molecule (4, 8).

The 8H8.1 mAb has been assigned to the CD7 cluster of differentiation during the 2nd International HLDA Workshop in Boston, U.S.A., in 1984 (WS Code: 38, Section T) (9).

The CD3 antigen is a complex of 5 polypeptidic chains: γ , δ , ϵ , ζ and η associated with the T-cell receptor (TCR) complex (10). The CD3 antigen is expressed by mature T lymphocytes and by a subset of thymocytes (11).

The UCHT1 mAb reacts with the ϵ chain of the CD3 complex (12). It has been assigned to the CD3 cluster of differentiation at the 1st International HLDA Workshop in Paris, France, in 1982 (WS Code: 3, Section T) (13).

CONJUGATION

Fluorescein isothiocyanate (FITC) is conjugated at 4–7 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

Multiparametric flow cytometry analysis of CD5, CD7, and CD3 antigen expression in hematopoietic neoplasia.

The coexpression of CD5, CD7 and CD3 antigen characterizes and identifies T-CLL/T-PLL (T-Chronic Lymphocytic Leukemia/T-Prolymphocytic Leukemia) (14–16).

The specific cytoplasmic expression of CD3 (cCD3 for cytoplasmic and CD3 for membraneous expression) on Precursor T-Lymphoblastic Lymphoma/Leukemia (CD5⁺CD7⁺mCD3⁻cCD3⁺) may differentiate this neoplasia from T-CLL / T-PLL (CD5⁺CD7⁺CD3⁻) (16).

The coexpression of CD5, CD7 and CD3 antigens may characterize and identify T-ALL (T-Acute Lymphoid Leukemia). Refer to complementary IO Test[®] 3 Conjugated Antibodies to confirm the immaturity of this neoplasia: HLA-DR-FITC / CD7-PE / CD45-ECD (PN IM3484), HLA-DR-FITC / CD34-PE / CD45-ECD (PN IM3478).

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.

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**BECKMAN
 COULTER™**

PN IM3484**25 tests****20 µL/test****CD5-FITC****CD7-PE****CD3-ECD****For Research Use Only. Not For Use In Diagnostic Procedures.****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 5×10^5 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 4 diagrams below are biparametric representations (Side Scatter versus Fluorescence Intensity or Fluorescence Intensity versus Fluorescence Intensity) of a CD3 negative T-CLL specimen (peripheral blood). Staining is with CD5-FITC / CD7-PE / CD3-ECD Conjugated Antibodies (PN IM3484). Lysis and fixation are with IOTest 3 Lysing Solution (PN IM3514) and IOTest 3 Fixative Solution (PN IM3515) respectively. All events acquired are shown on figure 1 and 2. Region A defines CD7 positive events then figured in dark on all figures. Figure 2 displays all events and defines the gating used by Region B (i.e. CD7^{bright}CD3⁻ events). Figures 3 and 4 show only events fulfilling conditions of region A x B.

Acquisition is with a COULTER® EPICS® XL™ flow cytometer equipped with System II™ Software. Analysis is with the EXPO™ Cytometer Software (PN 6605434).

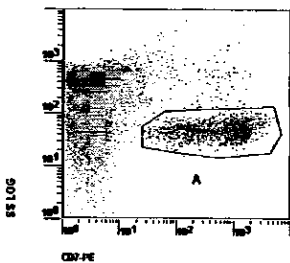


Figure 1

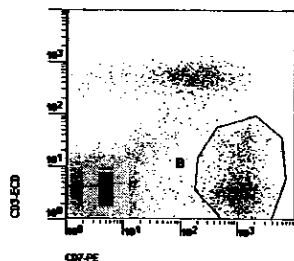


Figure 2

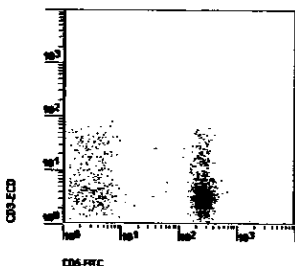


Figure 3

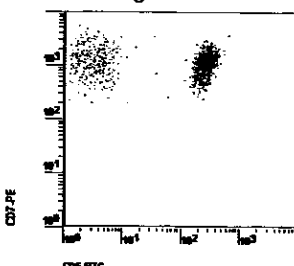


Figure 4

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MISCELLANEOUS

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