

**PN IM3486****25 tests****20 µL/test****CD25-FITC****CD4-PE****CD3-ECD**
**IO Test<sup>®</sup> 3**  
 Conjugated Antibodies

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

IO Test 3 Conjugated Antibodies – CD25-FITC / CD4-PE / CD3-ECD

PN IM3486 – 25 tests – 20 µL/test

	<b>CLONE 1</b>	<b>CLONE 2</b>	<b>CLONE 3</b>
<b>Specificity</b>	CD25	CD4	CD3
<b>Clone</b>	B1.49.9	13B8.2	UCHT1
<b>Hybridoma</b>	NS1 x Balb/c	NS1 x Balb/c	NS1 x Balb/c
<b>Immunogen</b>	Human alloactivated T lymphocytes (FC2)	Human thymocytes	Peripheral blood lymphocytes
<b>Ig Chain</b>	IgG2a	IgG1	IgG1
<b>Species</b>	Mouse	Mouse	Mouse
<b>Source</b>	Ascites fluid	Ascites fluid	Ascites fluid
<b>Purification</b>	Ion exchange or affinity chromatography	Ion exchange or affinity chromatography	Ion exchange or affinity chromatography
<b>Buffer</b>	2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide.		
<b>Conjugation</b>	Fluorescein isothiocyanate (FITC)	PE (R-Phycoerythrin)	ECD™ (Phycoerythrin-Texas-Red®-X)

**SPECIFICITY**

The CD25 molecule (known as Tac antigen and interleukine-2 receptor  $\alpha$  IL-2R $\alpha$ ) is expressed on resting CD4 positive lymphocytes and undetected on CD8 positive lymphocytes. However, all activated T lymphocytes express the CD25 protein. A subset of B lymphocytes (CD20 positive) expresses CD25 antigen. Granulocytes, monocytes, NK cells, platelets and erythrocytes do not express CD25 (1).

The B1.49.9 monoclonal antibody (mAb) has been assigned to the CD25 cluster of differentiation during the 2nd International Workshop on Human Leucocyte Differentiation Antigens (HLDA) in Boston, U.S.A., in 1984 (WS Code: T141, Section T) (2).

The CD4 molecule is a monomeric transmembrane glycoprotein expressed on a specific subset of peripheral blood T lymphocytes named "helper" T (Th) cells or T4 lymphocytes (3). It is expressed on the majority of the thymocytes, where it is frequently co-expressed with CD8 (4). CD4 is also expressed on non-T cells like the monocytes and the eosinophils. All the monocytes carry the CD4 antigen, although at a lower density than on T4 lymphocytes.

The 13B8.2 mAb has been assigned to the CD4 cluster of differentiation at the 3rd International HLDA Workshop in Oxford, England, in 1986 (WS Code: 501, Section T) (5).

The CD3 antigen is a complex of 5 polypeptidic chains:  $\gamma$ ,  $\delta$ ,  $\epsilon$ ,  $\zeta$  and  $\eta$  associated with the T-cell receptor (TCR) complex (6). The CD3 antigen is expressed by mature T lymphocytes and by a subset of thymocytes (7).

The UCHT1 mAb reacts with the  $\epsilon$  chain of the CD3 complex (8). 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) (9).

**CONJUGATION**

Fluorescein isothiocyanate (FITC) is conjugated at 7 – 10 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 CD25, CD4, and CD3 antigen expression in hematopoietic neoplasia.

Characterization and identification of T-CLL (Chronic Lymphocytic Leukemia) / T-PLL (Prolymphocytic Leukemia) / Mycosis fungoides and Sézary Syndrome / Adult T-cell leukemia-Lymphoma based on the expression of CD3 as a Pan-T marker together with CD4 and possibly CD25 antigen expression (10 – 12).

The CD25 activation marker is often expressed in leukemia / lymphoma derived from transformed lymphocytes (e.g. HTLV-1 infection on adult T-cell leukemia / lymphoma) (12).

**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™**

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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  $5 \times 10^5$  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.

**SELECTED RESEARCH REFERENCES**

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**MISCELLANEOUS**

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