

PN IM2667**50 tests
20 µL/test****CD3 - FITC****CD25 - PE****IO Test[®]**
Conjugated Antibodies

For Research Use Only. Not for use in diagnostic procedures.

SPECIFICITY

The two antigens are expressed simultaneously by a subpopulation of activated T cells.

CD3The CD3 antigen is a complex of 5 polypeptide chains: α , β , ϵ , ζ and η associated with the TcR. These chains are composed by a group of two invariant dimers, γ - ϵ and δ - ϵ associated with a variable dimer which consists of ζ homodimers, or ζ - η , or ζ - γ FcR heterodimers, or γ FcR homodimers (γ FcR is the γ chain of the Fc receptors) (1,2,3).

The CD3 complex associated with the TcR is involved in the recognition of peptides bound to the major histocompatibility complex I and II (MHC) during the immune response (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).

CD25

The molecular weight of the recognized antigen is 55 kDa.

The IL-2 receptor consists of at least three distinct membrane components: the α chain (IL-2R α , Tac, CD25), the β chain (IL-2R β , p75, CD122), and the γ chain (IL-2R γ). CD25 is undetectable on resting T cells but is induced upon T-cell activation.

B1.49.9 recognizes activated T and B cells. CD25 is absent on thymocytes, resting T and B cells, and NK cells. B1.49.9 does not inhibit the in vitro IL2 dependent proliferation.

B1.49.9 antibody was evaluated during the 3rd Workshop on Human Leucocyte Differentiation Antigens in Oxford (1986).

REAGENT

CD3	CD25
UCHT1	B1.49.9
IgG1 mouse	IgG2a
Peripheral blood lymphocytes	Human alloactivated T lymphocytes (FC2)

Source Ascites fluid

Purification Ion exchange or affinity chromatography

Conjugations FITC: Fluorescein isothiocyanate (FITC) is conjugated at 3 - 6 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

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

APPLICATION

Flow cytometry.

Double labeling with both anti-CD3 and anti-CD25 gives the ratio of T cells and activated T cells.

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.

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 20 - 25 °C prior to use.

PROCEDURE

This reagent is designed for Flow Cytometry.

Assay volume: 20 µL/5 x 10⁵ cells / test or 100µL whole blood.

A wash is required to yield optimal results.

EXAMPLE DATA

The graphs below are biparametric representations (Fluorescence Intensity versus Fluorescence Intensity) of lyzed normal human whole blood sample. Staining is with CD3-FITC / CD25-PE dual color reagent (PN IM2667) gated on lymphocytes.

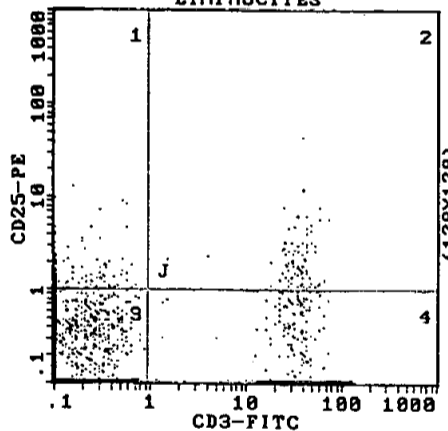
*Upper left quadrant (1) contains CD3- CD25+ lymphocytes representing non T lymphocytes expressing the CD25 molecule.

*Upper right quadrant (2) defines double positive CD3+ CD25+ lymphocytes representing T lymphocytes expressing the CD25.

*Lower left quadrant (3) contains double negative CD3- CD25- PBL.

*Lower right quadrant (4) contains CD3+ CD25- lymphocytes representing resting T lymphocytes.

Acquisition is with a COULTER R EPICS R XL flow cytometer. Analysis is with the XL SYSTEM II TM software.

LYMPHOCYTES

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**COULTER**

PARTNERS IN CELL ANALYSIS

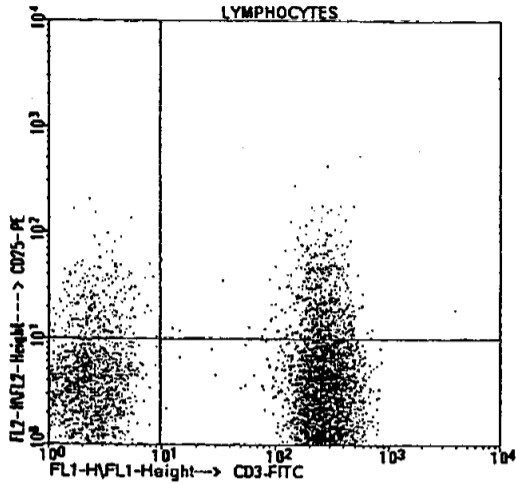
2667EX260198 01/02/98 AC-97260

**IMMUNOTECH**
A COULTER COMPANY

PN IM2667 CD3 - FITC
 50 tests CD25 - PE
 20 µL/test

For Research Use Only. Not for use in diagnostic procedures.

Acquisition is with a Becton Dickinson FACScan TM flow cytometer.
 Analysis is with the LYSYS II TM software.



SELECTED RESEARCH REFERENCES

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