IOTest® CD3-FITC / CD25-PE

PN IM2667U – 1 mL Liquid – 20 µL / test*

Analyte Specific Reagent.

Analytical and performance characteristics are not established.

REAGENT COMPONENTS

	Specifications of constituent 1	Specifications of constituent 2
Specificity	CD3	CD25
Clone	UCHT1	B1.49.9
Hybridoma	NS1 x Balb/c	NS1 x Balb/c
Immunogen	Peripheral blood lymphocytes	Human alloactivated T lymphocytes (FC2)
Ig Chain	IgG1	IgG2a
Species	Mouse	Mouse
Source	Ascites fluid	Ascites fluid
Purification	Ion exchange or affinity chromatography	Ion exchange or affinity chromatography
Conjugation	FITC (Fluorescein isothiocyanate)	PE (Phycoerythrin)
Molar Ratio	FITC / Ig: 3 – 6	PE / Ig: 0.5 – 1.5
λ Excitation range	468 – 509 nm	486 – 580 nm
λEmission range	504 – 541 nm (green)	568 – 590 nm (Orange)
Buffer	Buffer (PBS pH 7.2) plus 2 mg / mL BSA and 0.1% NaN_3	

SPECIFICITY

The CD3 antigen is a complex of 5 polypeptide chains: γ, δ, ε, ζ and η associated with the T-cell receptor (TCR) complex (1). The CD3 chains are clustered in 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/γFcR heterodimers (γFcR being the γ chain of the Fc receptors), or γFcR homodimers (1-3). The CD3 complex associated with the TCR is involved in the recognition of peptides bound to the major histocompatibility complex (MHC) class I and II, during the immune response (4).

The CD3 antigen is expressed by mature T lymphocytes and by a subset of thymocytes (5).

The UCHT1 monoclonal antibody reacts with the ε chain of the CD3 complex (6). It has been assigned to the CD3 cluster of differentiation at the 1st International Workshop on Human Leucocyte Differentiation Antigens in Paris, France, in 1982 (7).

The high affinity IL-2 receptor (IL-2R) is a trimeric complex composed of three polypeptides chains, α (IL-2Rα, Tac, p55, or CD25), β (IL-2Rβ, p75, or CD122), and γ (IL-2Rγ or p64). T lymphocytes express an intermediate-affinity IL-2 receptor that comprises β /γ or α/γ chain complex. IL-2Rβ and IL-2Rγ chains are involved in IL-2-mediated cellular signaling (8 – 10).

The CD25 molecule (known as Tac antigen and interleukine-2 receptor α IL-2Rα) is highly expressed on regulatory CD4-positive T lymphocytes and undetected on resting 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 (11).

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) (12).

REAGENT

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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. Do not use reagent beyond the expiration date on the vial label.
3. All specimens and samples must be considered as potentially infectious and must be handled with care (in particular: the wearing of protective gloves, gowns and goggles).
4. Do not expose reagents to strong light during storage or incubation.
5. Avoid microbial contamination of reagents or incorrect results might occur.
6. Avoid contact of samples with skin mucosa and eyes. Never pipet by mouth
7. Let it come to room temperature (18 – 25°C) before use.
8. Use general good laboratory practices when handling this reagent.

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.

EVIDENCE OF DETERIORATION

Any change in the physical appearance of this FITC- and PE-labeled combination (clear, yellowish-green to pinkish liquid) or any major variation in values obtained for control samples may indicate deterioration and the reagent should not be used.

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.

SELECTED RESEARCH REFERENCES

1. Thibault, G., Bardos, P., "Compared TCR and CD3 ε expression on αβ and γδ cells. Evidence for the association of two TCR heterodimers with three CD3 ε chains in the TCR/CD3 complex", 1995, J. Immunol.,154, 3814-3820,
2. Shores, E.W., Love, P.E., "TCR ζ a-chain in T cell development and selection", 1997, Curr. Opin. Immunol., 9, 380-389.
3. Ono, S., Ohno, H., Saito, T., "Rapid turnover of the CD3ζ chain independent of the TCR-CD3 complex in normal T cells", 1995, Immunity, 2, 639-644.
4. Julius, M., Maroun, C.R., Haughn, L., "Distinct roles for CD4 and CD8 as co-receptors in antigen receptor signalling", 1993, Immunol. Today, 4, 14, 177-183.

(*): 20 µL is the quantity of product sufficient to stain 5×10^5 cells in a standard immunofluorescence assay

I-IM2667U 2006-12-13

BECKMAN COULTER

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5. 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.
6. Tunnacliffe, A., Olsson, C., Traunecker, A., Krissansen, G.W., Karjalainen, K., De la Hera, A., "The majority of CD3 epitopes are conferred by the epsilon chain", 1989, Leucocyte Typing IV, White Cell Differentiation Antigens. W. Knapp, et al., Eds., Oxford University Press, 295-296.
7. Bernard, A., Brottier, 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., Springer Verlag, 9-135.
8. Sasaki, Y., Sugamura, K., "CD25 workshop panel report", 1996, Leucocyte Typing VI,White cell Differentiation Antigens, Kishimoto, T., et al, Eds., Garland Publishing, Inc., 802-804.
9. Callard, R.E., Gearing, A.J.H., "The cytokines and their receptors : Interleukins IL-2", 1994, The Cytokine FactsBook, Academic Press, 39-45.
10. Kaplan, D., "Autocrine secretion and the physiological concentration of cytokines", 1996, Immunol. Today, 17, 303-304
11. Sasaki, Y., Sugamura, K., "CD25 Workshop panel report", 1997, Leucocyte Typing VI, White Cell Differentiation Antigens. Kishimoto, T., et al, Eds., Garland Publishing, Inc., 802-804.
12. Haynes, B.F., "Summary of T cell studies performed during the second International Workshop and Conference on Human Leukocytes Differentiation Antigens", 1986, Leucocyte Typing II, Human T lymphocytes, Reinherz, E.L., et al. Eds., Springer-Verlag, 3-30.

PRODUCT AVAILABILITY

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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.
www.beckmancoulter.com

TRADEMARKS

Manufactured by:
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(*): 20 µL is the quantity of product sufficient to stain 5×10^5 cells in a standard immunofluorescence assay