IOTest® CD2-FITC / CD19-PE

PN IM1289U – 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	CD2	CD19
Clone	39C1.5	J3-119
Hybridoma	P3-X63-Ag.8.653 x LOU	NS1 x Balb/c
Immunogen	Human PHA-stimulated lymphocytic blasts	SKLY 18 cultured cells
Ig Chain	IgG2a	IgG1
Species	Rat	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: 5 – 7	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 CD2 antigen (for a review, see ref. 1) has been formerly described as the sheep E-rosette receptor and is alternatively known as T11 antigen or leucocyte function-associated molecule 2 (LFA-2). It is a 50 kDa single chain type I transmembrane glycoprotein that comprises two external domains belonging to the immunoglobulin superfamily (IgSF) (2). Crystallographic studies of soluble, deglycosylated forms of rat and human CD2 molecules (3), revealed that the N-terminal, distal domain 1 of the molecule is a V-type IgSF domain, and that the proximal domain 2 is a C-type IgSF domain. The V-type domain 1 lacks the usually conserved disulphide bonds between the beta sheets. The extracellular segment includes at least three N-glycosylation sites, believed to be involved in the interactions with the CD2 ligands. CD2 has a relatively large cytoplasmic domain which is required for the activation produced by certain combinations of CD2 antibodies (4).

Several epitopes can be distinguished on the CD2 molecule. The 39C1.5 monoclonal antibody (mAb), also known as CD2.9 (5), reacts with the T11-1 group of epitopes.

CD2 is present on all human non-B peripheral lymphocytes, on the majority of thymic T cells (6), and on a subset of thymic B cells.

The 39C1.5 mAb has been assigned to the CD2 cluster of differentiation at the 2nd International Workshop on Human Leucocyte Differentiation Antigens in Boston, USA, in 1984 (7).

The CD19 antigen is a single chain, type I integral membrane glycoprotein with a molecular weight of 95 kDa (8, 9). Its extracellular domain comprises 280 amino acids organized as two C2-type Ig-like domains separated by a smaller potentially disulfide-linked domain. The extensive cytoplasmic domain of CD19 contains nine conserved tyrosine residues, and several of these are located within potential src-homology region 2 (SH2)-binding sites. The CD19 molecule shows a full B lineage expression, from early pre-B cells to mature B lymphocytes. Its expression is lost during maturation to plasma cells (10, 11). The CD19 antigen is also expressed on the membrane of follicular dendritic cells and on most stabilized B cell lines. CD19 expression is not observed in normal T lymphocytes, NK cells, monocytes, and granulocytes (12).

The J3-119 mAb has been assigned to the CD19 cluster of differentiation at the 4th International Workshop on Human Leucocyte Differentiation Antigens in Vienna, Austria, in 1989 (8, 9).

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. Davis, S.J., Van Der Merwe, P.A., "The structure and ligand interactions of CD2 : implications for T-cell function", 1996, Immunol. Today, 4, 17, 177-187.
2. Sewel, W.A., Brown, M.H., Dunne, J., Owen, M.J., Crumpton, M.J., "Molecular cloning of the human T lymphocyte surface CD2 (T11) antigen", 1986, Proc.Natl. Acad.Sci. USA, 83, 8718-8722.

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

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BECKMAN COULTER

3. Bodian, D.L., Jones, E.Y., Harlos, K., Stuart, D.I., Davis, S.J., "Crystal structure of the extracellular region of the human cell adhesion molecule CD2 at 2.5 Å resolution", 1994, Structure, 8, 2, 755-766.
4. Thèze, J., Alzari, P.M., Bertoglio, J., "Interleukin 2 and its receptors: recent advances and new immunological functions", 1996, Immunol. Today, 10, 17,481-486.
5. Olive, D., Ragueneau, M., Cerdan, C., Dubreuil, P., Lopez, M., Mawas, C., "Anti-CD2 (Sheep red blood cell receptor) monoclonal antibodies and T-cell activation. Pairs of anti-T11-1 and T11-2 (CD2 subgroups) are strongly mitogenic for T cells in presence of 12-0 tetradecnoylphorbol 13 acetate", 1986, Eur. J. Immunol., 16, 1063-1068.
6. Bierber, B.E., Bogart, R.E., Wolff, I.H., Burkakoff, S.J., "Functional analysis of CD2 Mab reactivity", 1989, Leucocyte Typing IV, White Cell Differentiation Antigens. W. Knapp, et al., Eds., Oxford University Press, 274-277.
7. Haynes, B.F., "Summary of T cell studies performed during the second International Workshop and Conference on human leukocytes Differentiation Antigens", 1985, Leucocyte Typing II, Human T lymphocytes, Reinherz, E.L., et al. Eds., 3-30.
8. "CD Guide " Compiled by the organizing committee, 1989, Leucocyte Typing IV, White Cell Differentiation Antigens. W. Knapp, et al., Eds., Oxford University Press, 1078.
9. "Listing of all Fourth Workshop antibodies", 1989, Leucocyte Typing IV, White Cell Differentiation Antigens. W. Knapp, et al., Eds., Oxford University Press, 1094-1110.
10. Doody, G.M., Dempsey, P.W., Fearon, D.T., "Activation of B lymphocytes : integrating signals from CD19, CD22 and FcγRIIb1", 1996, Cur. Opin. Immunol., 8, 378-382.
11. Loken, M.R., Shah, V.O., Dattilio, K.L., Civin, C.I., "Flow cytometric analysis of human bone marrow. II. Normal B lymphocyte development", 1987, Blood, 70, 1316-1324.
12. Pesando, J. M., Bouchard, L. S., McMaster, B. E., "CD19 is functionally and physically associated with surface immunoglobulin", 1989, J. Exp. Med., 170, 2159-2164.

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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13276 Marseille Cedex 9, France

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