

**IOTest®
CD62L-FITC/
CD45RA-PE/CD8-PC7**

	CLONE 1	CLONE 2	CLONE 3
Specificity	CD62L	CD45RA	CD8
Clone	DREG56	2H4LDH11LDB9	SFC121Thy2D3
Hybridoma	SP2/0 x BALB/c	NS1 x BALB/c	NS1 x BALB/c
Immunogen	Peripheral blood lymphocytes	Peripheral blood lymphocytes	Peripheral blood lymphocytes
Ig Chain	IgG1	IgG1	IgG1
Species	Mouse	Mouse	Mouse
Source	Ascites fluid	Conditioned media	Conditioned media
Purification	Ion exchange or affinity chromatography	Affinity chromatography	Affinity chromatography
Fluorescence	Excites 468-509 nm / Emits 504-541 nm	Excites 486-580 nm / Emits 568-590 nm	Excites 486-580 nm / Emits 710-800 nm
Conjugation	FITC	PE	PC7
Molar Ratio	FITC/Ig: 4-6	PE/Ig: 0.5-1.5	PC7/Ig: 0.5-1.5

6607105 - 50 tests

PN 4238101-B



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

SPECIFICITY

The CD62L antigen (75-80kDa), also known as L-selectin, leucocyte adhesion molecule 1 (LAM-1), or lectin adhesion molecule 1 (LECAM-1)¹ was initially described using the mAb TQ1.² Among peripheral blood leucocytes, CD62L is constitutively expressed on resting neutrophils, eosinophils, basophils, monocytes, and important subsets of B- and CD4+ T- lymphocytes.^{1,4} It is weakly expressed on subsets of NK cells and CD8+ T-lymphocytes.² CD62L is also present, at various densities of expression, on bone marrow myeloid progenitors, including myeloblasts, promyelocytes^{1,5}, and on few thymocytes.^{1,2} Recent reports show that CD62L-positive memory CD4+ T lymphocytes play a role in isotype switching and induction of immunoglobulin production in naive B lymphocytes.⁶ Moreover, CD4+ T-helper (Th) lymphocytes can be distinguished into Th1 and Th2 subsets on the basis of differential expression of L-selectin, Th1 cells being CD62L-negative and Th2 cells being CD62L- positive.⁷ The DREG56 mAb was assigned to the cluster of differentiation during the 5th International workshop on Human Leukocyte Differentiation (HLDA) in Boston (1993).³ It was used as a reference mAb during the 6th International HLDA Workshop in Kobe, Japan in 1996.¹

The CD45 molecule (also named LCA for Leucocyte Common Antigen) groups single type I transmembrane glycoproteins with a molecular weight (MW) ranging from 180 to 220 kDa.^{8,9} The CD45 proteins contains three exons which encode peptide segments A, B, and C determinant, respectively.¹⁰ The differential splicing of the exons generates at least five isoforms, ABC, AB, BC, B and O.⁹ The CD45 family of proteins is expressed on the surface of all nucleated hematopoietic cells.¹⁰ The density of CD45 expression on lymphocytes is relatively constant and exceeds the density found on myelo-monocytes cells.⁹ Mixed expression of restricted form of CD45 among human peripheral T lymphocytes defines naive (or virgin or resting) CD45RA positive lymphocytes and memory (or primed or activated) CD45RO positive cells.¹¹ Furthermore, the percentage of CD45RO positive cells increases with aging.⁹ Among CD4+ T lymphocytes CD45RA positive cells comprise suppressor/inducer function, whereas the CD45RA negative subset includes helper cell function.^{9,12} The CD45RA expression on CD8+ lymphocytes may signal suppressor activity, whereas CD45RO phenotype may have specific cytotoxic activity. However, CD56+ NK cells express the CD45RA phenotype.⁹ In contrast to T lymphocytes, most peripheral B cells express the CD45RA isoform.^{9,10} 2H4LDH11LDB9 (2H4) mAb was evaluated during the 3rd and the 4th International workshop on Human Leukocyte Differentiation in Oxford (1986) and Vienna (1989) respectively. 2H4LDH11LDB9 (2H4) monoclonal antibody is restricted to the CD45RA antigen.^{13,14}

The CD8 molecule is a disulfide-linked dimer which exists either as a CD8 α homodimer or as a CD8 $\alpha\beta$ heterodimer. The CD8 antigen is expressed by the "cytotoxic/suppressor" T lymphocyte subpopulation (Tc cells) and with a lower density by a subset of NK cells.¹⁵ The majority of Tc cells express the CD8 molecule as an α/β heterodimer whereas NK cells are essentially CD8 $\alpha+\beta-$ (CD8 $\alpha+\beta+$).^{15,16}

REAGENTS

IOTest CD62L-FITC/CD45RA-PE/CD8-PC7
Conjugated Antibodies
PN 6607105 - 50 test - 20 μ L/test

BUFFER

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

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. Use Good Laboratory Practices (GLP) when handling reagent.
7. Harmful if swallowed.
8. After contact with skin, wash immediately with plenty of water.

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.

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

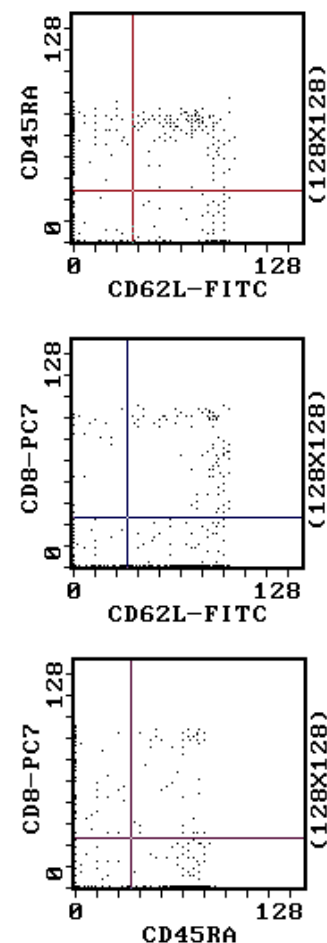
This reagent is designed for flow cytometry. Assay Volume; 20 μ L per 5 X 10⁵ cells in one test, or per 100 μ L of whole blood. A wash is required to yield optimal results.

EXAMPLE DATA

The histograms shown are representative of a normal EDTA whole blood sample, stained with

CD62L-FITC/CD45RA-PE/CD8-PC7 multicolor reagent (PN 6607105).

**Figures:
Acquisition with a COULTER® EPICS® XL™ and XL-MCL™ flow cytometer.**



SELECTED RESEARCH REFERENCES

1. Goda, K., Tanaka, T., Takeuchi, E., Miyasaka, M., "CD62L workshop panel report", 1997, Leucocyte Typing VI, 420-422.
2. Reinherz, E. L., Morimoto, C., Fitzgerald, K.A., Hussey, R.E., Daley, J.F., Schlossman, SF, "Heterogeneity of human T4+ inducer T cells defined by a monoclonal antibody that delineates two functional subpopulations", 1982, J. Immunol., 1, 128, 463-468.
3. Diacovo, T., Springer, T.A. "CD62L Cluster Report, 1995, Leucocyte Typing V - White Cell Differentiation

- Antigens. Schlossman, S.F., et al., Eds., Oxford University Press, 1503-1504.
4. Haddock, T.F., Fomovskaia, G.N., Davenport, R.D., "CD62L workshop: expression of L-selectin on leucocytes during short-term storage of whole blood", 1995, Leucocyte Typing V, 422-423.
 5. Griffin, J.D., Spertini, O., Ernst, T.J., Belvin, M.P., Levine, H.B., Kanakura, Y., Tedder, T.F., "Granulocyte-macrophage colony-stimulating factor and other cytokines regulate surface expression of the leukocyte adhesion molecule-1 on human neutrophils, monocytes and their precursors" 1990, J. Immunol. 145 2 576-584
 6. Tsuji, T., Nibu, R., Iwai, K., Kanegane, H., Yachie, A., Seki, H., Miyawaki, T., Taniguchi, N., "Efficient induction of immunoglobulin production in neonatal naive B cells by memory CD4+ T cell subset expressing homing receptor L-selectin", 1994, J. Immunol., 152, 4417-4424.
 7. Kanegane, H., Kasahara, Y., Niida, Y., Yachie, A., Sugii, S., Takatsu, K., Taniguchi, N., Miyawaki, T., "Expression of L-selectin (CD62L) discriminates Th1- and Th2- like cytokine-producing memory CD4+ T cells", 1996, Immunol., 87, 186-190.
 8. Weiss, L.M., Arber, D.A., Chang, K.L., "CD45 : a review", 1993, Applied Immunohistochemistry, 3, 1, 166-181.
 9. Poppema, S., Lai, R., Visser, L., Yan, X.J., "CD45 (Leucocyte Common Antigen) expression in T and B lymphocyte subsets", 1996, Leukemia and Lymphoma, 20, 217-222.
 10. Sewell, W.A., Cooley, M.A., Hegen, M., "CD45 Workshop Panel Report", 1997, Leucocyte Typing VI, White Cell Differentiation Antigens, 499-502.
 11. Gruber, R., Reiter, C., Riethmüller, G., "Triple immunofluorescence flow cytometry, using whole blood, of CD4+ and CD8+ lymphocytes expressing CD45RO and CD45RA", 1993, J. Immunol. Methods, 163, 173-179.
 12. Morimoto, C., Letvin, N.L., Distaso, J.A., "The isolation and characterization of the human suppressor inducer T cell subset", 1985, J. Immunol., 3, 134, 1508-1515.
 13. Schlossman, S.F., Morimoto, C., Streuli, M., Anderson, P., Rudd, C., "Surface structures defining functional T-cell subsets", 1989, Leucocyte Typing IV, White Cell Differentiation Antigens, 1070-1073.
 14. Streuli, M., Morimoto, C., Schrieber, M., Schlossman, S.F., Saito, H., "Characterization of CD45 and CD45R monoclonal antibodies using transfected mouse cell lines that express individual human leukocyte common antigens", 1988, J. Immunol., 11, 141, 3910-3914.
 15. Terry, L.A., DiSanto, J.P., Small, T.N., Flomenberg, N. "Differential expression and regulation of the human CD8alpha and CD8beta chains", 1990, Tissue Antigens 35, 82-91.
 16. Moebius, U., Kober, G., Griscelli, A.L., Hercend, T., Meuer, S.C., "Expression of different CD8 isoforms on distinct human lymphocyte subpopulations", 1991, Eur. J. Immunol., 21, 1793-1800

TRADEMARKS

Beckman Coulter Logo, IOTest, COULTER, EPICS, XL, and XL-MCL, are registered trademarks of Beckman Coulter, Inc.

For additional information call:
USA 1-800-526-7694
Outside the USA, contact your local
Beckman Coulter representative.

Beckman Coulter, Inc.
4300 N. Harbor Blvd.
Fullerton, CA 92835
www.beckmancoulter.com

Printed in USA
Made in USA

© 2002 Beckman Coulter, Inc.
All Rights Reserved.