

IOtest®
CD62L-FITC/
CD45RA-PE/CD4-PC7

| | CLONE 1 | CLONE 2 | CLONE 3 |
|---------------------|---|---------------------------------------|---------------------------------------|
| Specificity | CD62L | CD45RA | CD4 |
| Clone | DREG56 | 2H4LDH11LDB9 | SFC112T4D11 |
| 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 |

6607104 - 50 tests

PN 4238103-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, on eosinophils, basophils, monocytes, and on 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 promyelocytes¹⁵, 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.⁸ 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) CD45R0 positive cells.¹¹ Furthermore, the percentage of CD45R0 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 CD45R0 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.^{18,19}

The CD4 molecule (62kDa) is a monomeric transmembrane glycoprotein expressed on a specific subset of peripheral blood T lymphocytes named "helper" T (Th) cells or T4 lymphocytes.¹³ It is expressed on the majority of the thymocytes, where it is frequently co-expressed with CD8.¹⁴ CD4 is also expressed on non-T cells like monocytes and eosinophils. All the monocytes carry the CD4 antigen, although at a lower density than on T4 lymphocytes. Human CD4+ T lymphocytes can be divided into distinct and largely reciprocal subsets based on their differential expression of the CD45 isoforms CD45RA and CD45R0.¹⁵ The switch of expression from CD45RA (naive marker) to CD45R0 (memory marker) is one of the main hallmarks of the maturation of T lymphocyte-mediated immune response as a function of age and is correlated with the ability of T4 lymphocytes to express CD154, the CD40 ligand.¹⁶ Memory phenotype CD45R0+ T4 lymphocytes can be either CD62L+ or CD62L-.¹⁷ After stimulation with antigen in vitro, the CD62L+ cells synthesize mainly IL-4 and IL-5 cytokines, whereas the CD62L- cells produce IFN-γ, suggesting that these two subsets of memory CD4+ T lymphocytes resemble Th2-like and Th1-like cells respectively.⁷

REAGENTS

IOtest CD62L-FITC/CD45RA-PE/CD4-PC7
 Conjugated Antibodies
 PN 6607104 - 50 test - 20 µL/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.

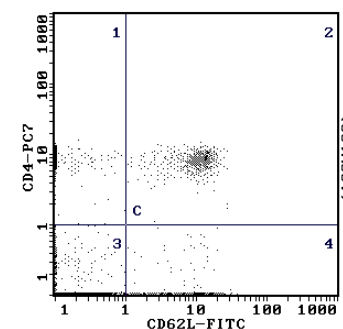
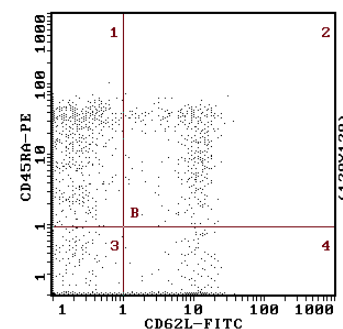
PROCEDURE

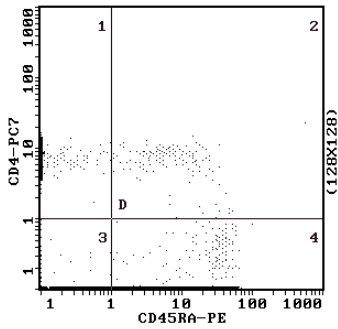
This reagent is designed for flow cytometry. Assay volume; 20 µL per 5 x 10⁵ cells in one test, or per 100 µL whole blood. A wash is required to yield optimal results.

EXAMPLE DATA

The histograms shown are representative of a normal EDTA whole blood sample, stained with CD62L-FITC/CD45RA-PE/CD4-PC7 multicolor reagent (PN 6607104).

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





SELECTED RESEARCH REFERENCES

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