

PN IM2713**CD62L - ECD****(DREG56)****IO Test®**

Conjugated Antibodies

100 tests
10 µL/test
For Research Use Only. Not for use in diagnostic procedures.
SPECIFICITY

The CD62L antigen, 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). CD62L is a type I integral membrane glycoprotein that belongs to the selectin family. It is composed of an amino-terminal C-type carbohydrate-binding lectin-like domain, an epidermal growth factor (EGF)-like domain, two short consensus repeat (SCR) sequences similar to those found in complement-binding proteins, a transmembrane region, and a short cytoplasmic region (3). The extracellular membrane-proximal 15 residue region is critical for proteolytic release from cell surface (1).

Among the 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, promyelocytes, granulocyte-macrophage colony forming unit (CFU-GM), as well as on burst-forming unit erythroid (BFU-E) (1,5), and on few thymocytes (1,2).

CD62L shows a 65 / 74 kDa apparent molecular weight (Mr) under non-reducing / reducing conditions, when characterized from lymphocytes. It shows a 95 kDa Mr under reducing conditions when characterized from neutrophils (1).

CD62L mediates the binding of lymphocytes to the specialized high endothelial cells present in postcapillary venules (HEV) of lymph nodes and is involved in lymphocyte homing to HEV. CD62L is also involved in leucocyte rolling to endothelial cells at sites of injury or inflammation. Furthermore, CD62L plays a role in the neutrophil CD18-dependent arrest (6).

Evidences indicate that L-selectin may also act as a signaling molecule, and may trigger neutrophil activation via the Mac-1 (CD11b/CD18) β2-integrin (7,8).

CD62L expression on neutrophils is considerably downmodulated by *in vitro* phorbol myristate acetate (PMA) stimulation (9). Studies indicate that the CD50 molecule (ICAM-3) should play a role in the physiological downmodulation of the surface expression of CD62L on neutrophils (10). On the other hand, CD45 engagement is reported to induce CD62L downregulation on lymphocytes but not on neutrophils (11). Other reports suggest that downregulation of CD62L could be due to a protease dependent shedding mechanism (9,12).

Phorbol myristate acetate (PMA) stimulation of purified peripheral blood lymphocytes results in downmodulation of L-selectin expression (3). *In vivo* activated tonsil B lymphocytes display reduced levels of CD62L. In contrast, the expression of CD62L is highly increased on *in vitro* activated B lymphocytes when the cells are stimulated for four days with CD40 monoclonal antibody (mAb) plus interleukin (IL)-4 (13). Cord blood CD5-negative B lymphocytes, in contrast to CD5-positive ones, express CD62L at levels much lower than those found on normal adults (14).

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 (15). 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 (16).

Studies on the expression of monocyte surface markers after cell preparation procedures have shown that Ficoll purification, as well as various leucocyte isolation procedures, may be responsible of artefactual changes in the expression of CD62L (11,17,18), and that buffy coat cell preparations may be recommended. If a lysing process is to be used, ammonium chloride mediated lysis performed at 4 °C, using EDTA treated blood, should provoke the smallest possible alterations of the prepared monocytes (17).

The Dreg56 mAb was originally raised against rapidly shed molecules released from the cell surface of activated human leucocytes (19). It was shown to recognize an epitope included in the lectin-like distal domain of the CD62L antigen (20). It is particularly effective in blocking CD62L-mediated lymphocyte binding to lymph node HEV from peripheral but not mucosal lymphoid tissues (19), as well as neutrophil binding to ELAM-1 transfected fibroblasts (21), or rolling along the venular wall (22). This antibody has been assigned during the Vth International Workshop on Human Leucocyte Differentiation Antigens (Vth HLDA) in Boston, 1993 (3). It was used as a reference mAb (Ref.33) during the Vth HLDA in Kobe, 1996 (1).

REAGENT

Clone	DREG56
Isotype	IgG1 mouse
Immunogen	Activated human leucocytes
Hybridoma	SP2/0 x Balb/c spleen cells
Source	Ascites fluid
Purification	Ion exchange or affinity chromatography
Conjugation	ECD: The Ig is conjugated to a tandem dye constituted of R-phycoerythrin covalently linked to texas red at 0.8-1 mole of ECD per mole of Ig.
Excitation wavelength:	488 nm
Maximum emission wavelength:	613 nm
Main emission color:	Red
Buffer	2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide.

APPLICATION**Flow cytometry**

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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.

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**COULTER**

PARTNERS IN CELL ANALYSIS

**IMMUNOTECH**
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2713EX040698 04/06/98 AC-98115

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- 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.
- Never pipet by mouth and avoid contact of samples with skin and mucous membranes
- Do not use antibody beyond the expiration date on the label.
- Do not expose reagents to strong light during storage or incubation.
- 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: 10 µL/5 x 10⁵ cells / test or 100µL whole blood.

This reagent works with a wash procedure. COULTER (R) Q-Prep (TM) Workstation with ImmunoPrep (TM) Reagent System is usable when a wash step is performed at the end of procedure.

EXAMPLE DATA

The graph below is a biparametric representation (Fluorescence Intensity versus Fluorescence Intensity) of a lyzed normal whole blood sample. Staining is with 10 µL of CD62L-ECD monoclonal antibody (PN IM2713), to which 10 µL of CD8-FITC/CD4-PE (CYTO-STAT combination PN 6603802) were added. Lysis is run on a Multi-Q-Prep Workstation, using the ImmunoPrep Reagent System (PN 7546999) with a wash step.

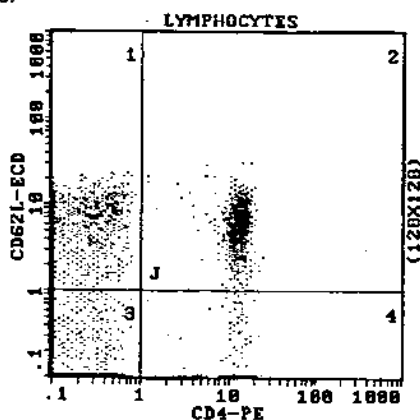
Acquisition is with a COULTER R EPICS R XL TM flow cytometer. Analysis is with the XL SYSTEM II TM software. Gate is on the lymphocytes. The quadrant statistic cursors are set using a combination of the following isotypic controls (10 µL PN IM2714 + 10 µL PN 6603796).

*Upper-left quadrant (1) contains CD62L-positive, CD4-negative lymphocytes.

*Upper-right quadrant (2) contains CD4, and CD62L double positive T lymphocytes.

*Lower-left quadrant (3) contains CD4 and CD62L double negative events.

*Lower-right quadrant (4) contains CD62L-negative, CD4-positive T lymphocytes.



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2713EX040698 04/06/98 AC-98115

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