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# TECHNICALLY *Speaking*

Place your order with CEDARLANE® or your local distributor.

*Please contact CEDARLANE® for lot specific information.*

## **FITC Anti-Rat CD62L Monoclonal Antibody**

**CL085F**

**CL085F-5**

**LOT: 8531**

### **DESCRIPTION:**

Cedarlane's FITC anti-rat CD62L monoclonal antibody recognizes rat leukocyte selectin (L-selectin). CD62L is expressed constitutively on leukocytes and is expressed by a subset of thymocytes predominantly single positive CD4 or CD8 cells and a majority of lymph node cells in rats. CD62L binds well to O-glycans present on certain glycoforms of the mucins GlyCAM-1, CD34 and MAdCAM-1. CD62L is reported to be capable of reported to be capable of transducing signals when cross-linked and has been shown to stimulate  $\beta_2$ -integrin-mediated adhesion when bound to soluble GlyCAM-1. [1]

L-selectin expressing CD4<sup>+</sup>CD8<sup>-</sup> thymocytes were shown through adoptive transfer to protect rats from insulin-dependent diabetes. CD4<sup>+</sup>CD8<sup>-</sup> thymocytes must mature to the stage of L-selectin expression before they can mediate normal T cell function. [2]

Sensitive detection systems may be required on some tissues as expression of rat CD62L is weak.

Applications include: Flow cytometry and ELISA [1].

### **PRESENTATION:**

100 $\mu$ g (CL085F) or 500 $\mu$ g (CL085F-5) FITC conjugated Ig buffered in PBS, 0.02% NaN<sub>3</sub> and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml.

For more information or to place an order please contact...

**CEDARLANE®**  
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**STORAGE/STABILITY:**

Store at 4°C. For long term storage, aliquot and freeze unused portions at -20°C in volumes appropriate for single usage. Avoid freeze/thaw cycles.

**SPECIFICATIONS:**

Clone: MRC OX-85

**Hybridoma Production:**

Immunization: Immunogen: Recombinant rat L-selectin  
Donor: BALB/c Spleen  
Fusion Partner: NSI/1

Specificity: Rat Leukocyte Common Antigen (L-selectin)

Ig Class: Mouse IgG<sub>1</sub>

Presentation: FITC conjugated Ig buffered in PBS, 0.02% NaN<sub>3</sub> and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml. (Purified from ascitic fluid via Protein G Chromatography)

Antibody Concentration: 0.1 mg/ml

**FLOW CYTOMETRY ANALYSIS:****Method:**

1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population with Lympholyte®-Rat cell separation medium (CL5040).
2. Wash 2 times.
3. Resuspend the cells to a concentration of  $2 \times 10^7$  cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain  $1 \times 10^6$  cells, representing 1 test).
4. To each tube, add 50µl of a 0.5 – 1.0 µg\* dilution of **CL085F** or **CL085F-5** per  $10^6$  cells.
5. Vortex the tubes to ensure thorough mixing of antibody and cells.
6. Incubate the tubes for 30 minutes at 4°C.  
(It is recommended that the tubes are protected from light, since most fluorochromes are light sensitive.)
7. Wash 2 times at 4°C.
8. Resuspend the cell pellet in 50 µl ice cold media B.
9. Transfer to suitable tubes for flow cytometric analysis containing 15 µl of propidium iodide at 0.5 mg/ml in PBS. This stains dead cells by intercalating in DNA.

Media:

- A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100  $\mu$ l of 2 M sodium azide in 100 mls.)
- B. Phosphate buffered saline (pH 7.2) + 0.5% bovine serum albumin + sodium azide (100  $\mu$ l of 2 M sodium azide in 100 mls.)

Results:Tissue Distribution by Flow Cytometry Analysis:

Rat Strain: Wistar

Cell Concentration:  $1 \times 10^6$  cells per test

Antibody Concentration: 1.0  $\mu$ g /  $10^6$  cells

Cell Source: Thymus

Cell population

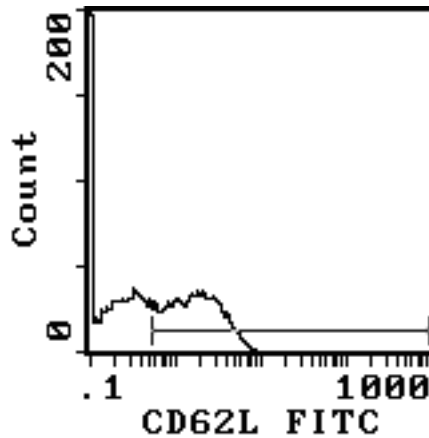
Whole thymus

CD4<sup>+</sup>CD8<sup>-</sup>

Percentage of cells stained above control:

16.0%

51.2%



Cell Source: CD4<sup>+</sup>CD8<sup>-</sup>

Percentage of cells stained above control: 51.2%

**N.B. Appropriate control samples should always be included in any labelling studies.**

**\* For optimal results in various applications, it is recommended that each investigator determine dilutions appropriate for individual use.**

**REFERENCES:**

1. Nicholson, M.W., Barclay, A.N., Singer, M.S., Rosen, S.D., and P.A. Van der Merwe. (1997) Affinity and Kinetic Analysis of L-Selectin (CD62L) binding to glycosylation-dependent Cell-Adhesion Molecule-1. *J. Biol. Chem.* 273: 763-770.
2. Seddon, B., Abdelhadi, S., Nicholson, M., and D. Mason. (1996) CD4<sup>+</sup>CD8<sup>-</sup> thymocytes that express L-selectin protect rats from diabetes upon adoptive transfer. *Eur. J. Immunol.* 26: 2702-2708.

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