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TECHNICALLY Speaking

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

PE Anti-Rat CD62L Monoclonal Antibody

CL085PE
CL085PE-4
LOT: 8551

DESCRIPTION:

Cedarlane's PE 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 capable of transducing signals when cross-linked and has been shown to stimulate β_2-integrin-mediated adhesion when bound to soluble GlyCAM-1. [1]

L-selectin expressing $CD4^+CD8^-$ thymocytes were shown through adoptive transfer to protect rats from insulin-dependent diabetes. $CD4^+CD8^-$ 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:

50µg (CL085PE) or 200µg (CL085F-4) FITC conjugated Ig buffered in PBS, 0.02% NaN_3 and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml.

STORAGE/STABILITY:

Store at +4°C. **DO NOT FREEZE**. Avoid prolonged exposure to light.

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

CEDARLANE® LABORATORIES LIMITED

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in North America

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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

Ig Class: Mouse IgG_1

Presentation: PE conjugated Ig buffered in PBS, 0.02% NaN_3 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 $2x10^7$ cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain $1x10^6$ cells, representing 1 test).
4. To each tube, add 50µl of a 1.0 – 0.5µg* dilution of **CL085PE** or **CL085PE-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.

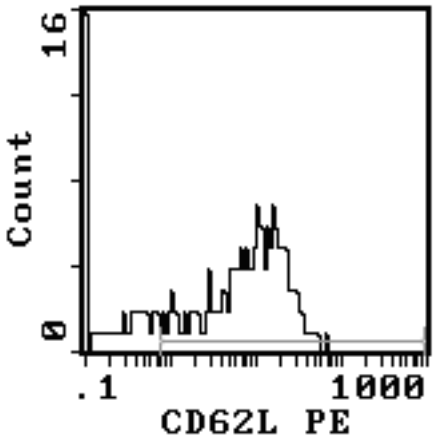
Media:

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

Results:

Tissue Distribution by Flow Cytometry Analysis:
Rat Strain: Wistar
Cell Concentration: $1x10^6$ cells per test
Antibody Concentration: 1.0 µg /10^6 cells
Cell Source: Thymus

Cell population	Percentage of cells stained above control:
Whole thymus	13.9%
$CD4^+CD8^-$	66.2%



Cell Source: $CD4^+CD8^-$
Percentage of cells stained above control: 66.2%

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.**

R-Phycoerythrin conjugates are produced under license and protected under Stanford University held patents 4,520,110; 4,542,104; 4,859,582; 5,055,556 (U.S.): 76695 (EPC): 548440 (Australia): 1,179,942 (Canada): and 1,594,827 (Japan).

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-Selctin (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) $CD4^{+}CD8^{-}$ thymocytes that express L-selectin protect rats from diabetes upon adoptive transfer. Eur. J. Immunol. 26: 2702-2708.

FOR RESEARCH USE ONLY

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