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TECHNICALLY Speaking

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

Purified Anti-Rat CD62L Monoclonal Antibody

CL085AP
CL085AP-2
LOT: 8521

DESCRIPTION:

Cedarlane's purified 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 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:
250µg (CL085AP) or 500µg (CL085AP-2) purified Ig buffered in PBS and 0.02% NaN_3.

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

CEDARLANE® LABORATORIES LIMITED
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or visit our website for a list of our international distributors including contact information
website: www.cedarlanelabs.com • e-mail: info@cedarlanelabs.com

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_1

Presentation: Purified Ig buffered in PBS with the addition of 0.02% NaN_3 (Purified from ascitic fluid via Protien G Chromatography).

Antibody Concentration: 1.0mg/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 **CL085AP** or **CL085AP-2** 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.
7. Wash 2 times at 4°C.
8. Add 100 µl of secondary antibody **CLCC30204** (PE Goat anti-mouse IgG (H+L)) at 1:500 dilution.
9. Incubate the tubes at 4°C for 30-60 minutes.
   (It is recommended that the tubes are protected from light since most fluorochromes are light sensitive).
10. Wash 2 times at 4°C in media B.

11. Resuspend the cell pellet in 50 µl ice cold media B. 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:

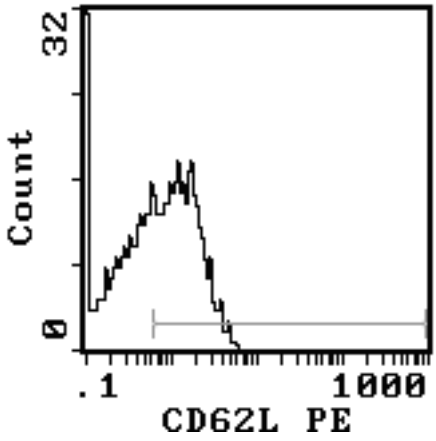
A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 µl of 2 M sodium azide in 100 mls.)
B. Phosphate buffered saline (pH 7.2) + 0.5% bovine serum albumin + sodium azide (100 µl of 2 M sodium azide in 100 mls.)

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	10.7%
$CD4^+CD8^-$	48.9%



Cell Source: $CD4^+CD8^-$
Percentage of cells stained above control: 48.9%

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-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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