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TECHNICALLY *Speaking*

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

Biotin Anti-Mouse CD62L Monoclonal Antibody

CL8918B

CL8918B-3

LOT: 1841

DESCRIPTION:

Cedarlane's anti-mouse CD62L (L-selectin Ly 22) monoclonal antibody reacts with a 90 kDa protein which is involved with the homing of lymphocytes to peripheral lymph nodes.

L-selectin is expressed on most T and B lymphocytes, neutrophils, monocytes and eosinophils.¹ Pre-incubation of lymphocytes with this antibody completely and specifically blocks binding of lymphocytes to high endothelial venules (HEV) in vitro^{2,3,6} and the migration of lymphocytes to lymph nodes in vivo.^{2,3} Polymorphonuclear cells preincubated with this antibody do not migrate to the inflammatory foci.³

Applications of CL8918B include flow cytometry^{3,4,5} and IHC.⁴

PRESENTATION:

100 µg (CL8918B) or 300 µg (CL8918B-3) Biotin conjugated Ig buffered in PBS, 0.02% NaN₃ and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml.

STORAGE/STABILITY:

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

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

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SPECIFICATIONS:

Clone: MEL-14

Hybridoma Production:

Immunization: Immunogen: Mouse B cell Lymphoma, 38C-14
Donor: Fischer Rat Spleen

Fusion Partner: P3 X 63Ag8.653

Specificity: Mouse CD62L (L-selectin Ly 22)

Ig Class: Rat IgG_{2a}

Format: Biotin conjugated Ig buffered in PBS, 0.02% NaN₃ 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[®]-M cell separation medium (CL5030).
2. Wash 2 times.
3. Resuspend the cells to a concentration of 2×10^7 cells/ml in media A. Add 50 μ l of this suspension to each tube (each tube will then contain 1×10^6 cells, representing 1 test).
4. To each tube, add 0.1-0.2 μ g* of **CL8918B** or **CL8918B-3** 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 μ l of secondary antibody **CLCSA1001** (Streptavidin-FITC) at a 1:500 dilution.
9. Incubate tubes at 4°C for 30 - 60 minutes (It is recommended that tubes are protected from light since most fluorochromes are light sensitive).
10. Wash 2 times at 4°C.
11. Resuspend the cell pellet in 50 μ l ice cold media B.
12. Transfer to suitable tubes for flow cytometric analysis containing 15 μ l 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 μ l of 2M sodium azide in 100 mls).
- B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 μ l of 2M sodium azide in 100 mls).

Results:Tissue Distribution by Flow Cytometry Analysis:

Mouse Strain: BALB/c

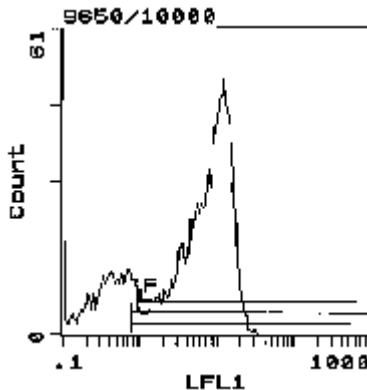
Cell Concentration : 1×10^6 cells per test

Antibody Concentration Used: 0.2 μ g/ 10^6 cells

Isotypic Control: Biotin Rat IgG_{2a}

Cell SourcePercentage of cells stained above control:

| | |
|------------|-------|
| Thymus | 59.8% |
| Spleen | 66.3% |
| Lymph Node | 75.8% |



Cell Source: Lymph Node

Percentage of cells stained above control: 75.8%

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) Fink, P., W. Gallatin, R. Reichert, *et al.* 1985. Homing receptor-bearing thymocytes, an immunocomponent cortical subpopulation. *Nature* 313: 233-235
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- 3) Lewinsohn, D.M., R.F. Bargatze, E.C. Butcher 1987. Leukocyte-endothelial cell recognition: evidence of a common molecular mechanism shared by neutrophils, lymphocytes and other leukocytes. *J.Immunology* 138:4313-4321
- 4) Reichert, R., M. Gallatin, E. Butcher, *et al.*. 1984. A homing receptor-bearing cortical thymocyte subset: Implications for thymus cell migration and the nature of cortisone-resisatant thymocytes. *Cell* 38: 89-99
- 5) Siegelman, M., I.C. Cheng, I.L. Weissman, *et al.* 1990. The mouse lymph node homing receptor is identical with the lymphocyte cell surface receptor Ly-22: Role of the EGF domain in endothelial binding. *Cell* 61: 611-622
- 6) Jalkanen, S., R.F. Bargatze, J. Toyos *et al.* 1987. Lymphocyte Recognition of High Endothelium: Antibodies to Distinct Epitopes of an 85-95-kD Glycoprotein to Lymph Node, Mucosal, or Synovial Endothelial Cell. *J. of Cell Biol.* 105: 983-990

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