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Monoclonal Antibody to CD62L / L-Selectin - FITC

Alternate names: CD62 antigen-like family member L, LAM-1, LECAM1, LNHR, LYAM1, Leu-8, Leukocyte adhesion molecule 1, Leukocyte surface antigen Leu-8, Leukocyte-endothelial cell adhesion molecule 1, Lymph node homing receptor, SELL, TQ1, gp90-MEL

Catalog No.: CL032F

Quantity: 0.1 mg

Concentration: 0.1 mg/ml

Background: L-selectin is expressed on most T and B lymphocytes, neutrophils, monocytes, 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.

Uniprot ID: P18337

NCBI: NP_035476.1

GeneID: 20343

Host / Isotype: Rat / IgG2a

Clone: MEL-14

Immunogen: Mouse B cell Lymphoma, 38C-14
Donor: Fischer Rat Spleen
Fusion Partner: P3 X 63Ag8.653

Format: **State:** Liquid
Purification: Protein G Chromatography
Buffer System: PBS, 0.02% NaN3 and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml
Label: FITC

Applications: Flow cytometry.
Immunohistochemistry.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This monoclonal antibody reacts with a 90 kDa protein which is involved with the homing of lymphocytes to peripheral lymph nodes.
Species: Mouse.
Other species not tested.

Storage: Store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General References: 1) Fink, P., W. Gallatin, R. Reichert, et al. 1985. Homing receptor-bearing thymocytes, an immunocomponent cortical subpopulation. Nature 313: 233-235
2) Gallatin,W.M., I.L. Weissman., E.C. Butcher 1983. A cell surface molecule involved in organ specific homing of lymphocytes. Nature 304:30-34
3) Lewinsohn, D.M., R.F. Bargatze, E.C. Butcher 1987. Leukocyteendothelial cell recognition: evidence of a common molecular mechanism
shared by neutrophils, lymphocytes and other leukocytes. J.Immunology 138:4313-4321
4) Reichert, R., M. Gallitin, E. Butcher, et al.. 1984. A homing receptorbearing 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 Antigen Differentially Inhibit Lymphocyte Binding to Lymph Node, Mucosal, or Synovial Endothelial Cell. J. of Cell Biol. 105: 983-990

Protocols:

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.
2. Wash 2 times.
3. Resuspend the cells to a concentration of 2x10e7 cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1x10e6 cells, representing 1 test).
4. To each tube, add 0.2 - 0.5 µg* of this Ab per 10e6 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 flurochromes 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 2M sodium azide in 100 mls).
B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 µl of 2M sodium azide in 100 mls).

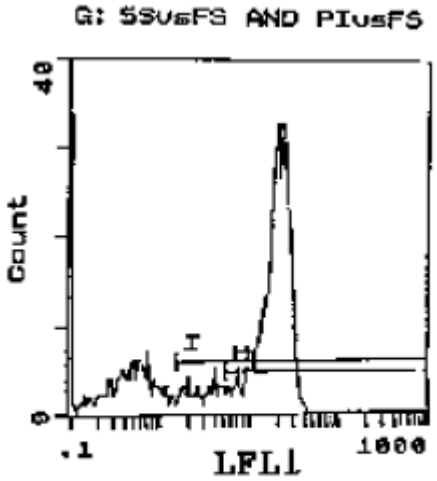
Results - Tissue Distribution by:
Mouse Strain: BALB/c
Cell Concentration: 1x10e6 cells per test
Antibody Concentration Used: 0.2 µg/10e6 cells
Isotypic Control: FITC Rat IgG2a

Cell Source: Percentage of cells stained above control:
Thymus: 88.8%
Spleen: 36.6%
Lymph Node: 74.4%

Strain Distribution:

Cell Concentration: 1x10e6 cells per test
Antibody Concentration Used: 0.2 µg/10e6 cells
Strains Tested: BALB/c, CBA/J, C3H/He, C57BL/6, AKR
Positive: BALB/c, CBA/J, C3H/He, C57BL/6, AKR
Negative: none

Pictures:



Cell Source: Lymph Node
Percentage of cells stained above control: 74.4%