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TECHNICALLY Speaking

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

Purified Anti-Mouse CD62L
No Azide, Monoclonal Antibody

CL8918NA
LOT: 1821NA

DESCRIPTION:

Cedarlane's anti-mouse CD62L (L-selectin) 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, 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 CL8918NA include flow cytometry,[3,4,5] IHC[4] and immunoprecipitation.[3,5,6]

PRESENTATION:

1 mg purified Ig buffered in PBS, no preservative, 0.2 µm filtered.

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. Should be handled under aseptic conditions.

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

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website: www.cedarlanelabs.com • e-mail: info@cedarlanelabs.com

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: Purified Ig buffered in PBS, no preservative. (Purifed from ascitic fluid via Protein G Chromatography).

Antibody Concentration: 1.0 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 $2x10^7$ 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 0.1-0.2 µg* of **CL8918NA**.
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 **CLCC40001** (FITC Goat anti-rat 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.
12. 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:

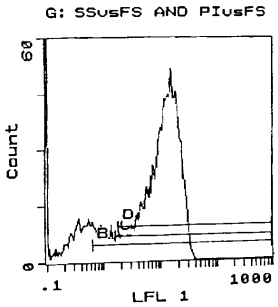
A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 µl of 2M sodium azide in 100 mls).
B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 µl of 2M sodium azide in 100 mls).

Results:

Tissue Distribution by Flow Cytometry Analysis:

Mouse Strain: BALB/c
Cell Concentration : $1x10^6$ cells per test
Antibody Concentration Used: 0.2 µg/10^6 cells
Isotypic Control: Rat IgG_{2a}

Cell Source	Percentage of cells stained above control:
Thymus	85.8%
Spleen	41.0%
Lymph Node	75.0%



Cell Source: Lymph Node
Percentage of cells stained above control: 75.0%

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.**

Strain Distribution by Flow Cytometry Analysis:

Procedure: see page 2
Tissue: Spleen
Cell Concentration: $1x10^6$ cells per test
Antibody Concentration Used: 0.2 µg/10^6 cells
Strains Tested: BALB/c, C57BL/6, C3H/He, CBA/J, AKR
Positive: BALB/c, C57BL/6, C3H/He, CBA/J, AKR
Negative: none

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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
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