

# Produktinformation



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Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

### Anti-Mouse CD62L (L-Selectin Ly 22) Monoclonal Antibody-Ascites

CL8918A LOT: 1811

#### **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, eosinophils. Pre-incubation of lymphocytes with this antibody completely and specifically blocks binding of lymphocytes to high endothelial venules (HEV) in vitro<sup>2,3,6</sup> and the migration of lymphocytes to lymph nodes in vivo. Polymorphonuclear cells preincubated with this antibody do not migrate to the inflammatory foci. <sup>3</sup>

Applications of CL8918A include flow cytometry, 3,4,5 immunohistochemistry 4 and immunoprecipitation. 3,5,6

#### PRESENTATION:

0.5 ml, lyophilized ascites

#### STORAGE/STABILITY:

Lyophilized form stable at 4°C or -20°C. Reconstitute with 0.5 ml of cold distilled water. After reconstitution, aliquot and freeze unused portions in volumes appropriate for single usage. Avoid repeated 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 IgG2a

Format: Ascitic fluid (lyophilized)

Antibody Concentration: 10.5 mg/ml (as determined by RID)

#### FLOW CYTOMETRY ANALYSIS:

#### Method:

- 1. Prepare a cell suspension in media A. For spleen cell preparations, deplete the red blood cell population with Lympholyte<sup>®</sup>-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  $\mu$ l of this suspension to each tube (each tube will then contain  $1x10^6$  cells, representing 1 test).
- 4. To each tube, add 50 µl of a 1/20,000 1/50,000 dilution\* of **CL8918A**.
- 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  $\mu$ 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 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:

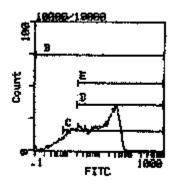
Mouse Strain: BALB/c

Cell Concentration: 1x10<sup>6</sup> cells per test

Antibody Concentration Used: 1/32,000 in 50 µl/106 cells

Isotypic Control: Rat IgG<sub>2a</sub>

Cell Source	Percentage of cells stained above control:
Thymus	64.1%
Spleen	65.2%
Lymph Node	75.1%



Cell Source: Lymph Node
Percentage of cells stained above control: 75.1%

# 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<sup>6</sup> cells per test Antibody Concentration Used: 1/5000 in 50 µl

Strains Tested: BALB/c, C57BL/6, C3H/He, CBA/J, AKR

Positive: BALB/c, C57BL/6, C3H/He, CBA/J, AKR

Negative: none

#### **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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- 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

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