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TECHNICALLY *Speaking*

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

Biotin Anti-Rat CD11a (LFA-1 α Chain) Monoclonal Antibody

CL017B

LOT:

DESCRIPTION:

LFA-1 (lymphocyte function associated molecule-1) is one of the leukocyte integrins. It is a heterodimer consisting of α and β subunits of 160-170 kDa and 95-100 kDa respectively.

LFA-1 promotes non-antigen dependent adhesion of T-cells to a variety of lymphoid cells that bear its complementary receptor I-CAM-1 (1). It has a broad distribution and is found on most common lymphocytes.

Cedarlane's CL017B is specific for the α subunit of LFA-1. It inhibits homeotypic aggregation of PHA blasts and blocks the binding of rat lymphocytes to purified rat ICAM-1 (1).

Applications include immunoprecipitation, flow cytometric analysis, immunohistochemistry, cryostat sections, and *in vivo/in vitro* functional studies (1,2,3).

PRESENTATION:

100 μ g, Biotin conjugated Ig buffered in PBS + 0.1% NaN_3 and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml.

STORAGE/STABILITY:

Stable at 4°C. For long term storage, aliquot and freeze unused portion at -20°C in volumes appropriate for single usage. Avoid repeated freeze /thaw cycles. Check label for expiry date.

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

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SPECIFICATIONS:

Clone: WT.1

Hybridoma Production:

Immunization: Immunogen: Rat Splenic PHA blasts
Donor: BALB/c spleen

Fusion Partner: Mouse myeloma cell line PAI

Specificity: Rat CD11a (LFA-1 α chain)

Ig Class: Mouse IgG_{2a}

Antibody Concentration: 0.1 mg/ml.

FLOW CYTOMETRY ANALYSIS:

Method:

1. Prepare 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 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 contains 1×10^6 cells representing 1 test).
4. To each tube add 1.0 μ g of **CL017B**.
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 1/700 dilution.
9. Incubate 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 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 phosphate buffered saline. (This stains dead cells by intercalating 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 Cytometric Analysis:

Rat Strain: Wistar

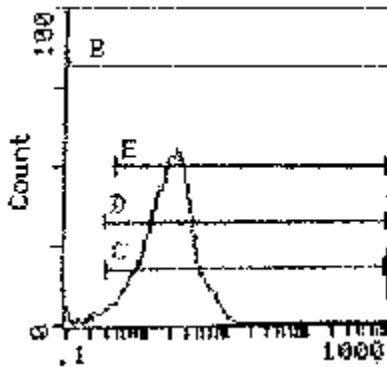
Cell Concentration: 1×10^6 cells per test

Antibody Concentration Used: $1.0 \mu\text{g}/10^6$ cells

Isotypic Control: Biotin Mouse IgG_{2a,k}

Cell Source Percentage of cells stained above control:

Thymus 95.3%

**LFL1**

Cell Source: Thymus

Percentage of cells stained above control: 95.3%

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. Tamatani, T., M. Kiotani and M. Miyasaka. 1991 Molecular mechanisms underlying lymphocyte recirculation II. Differential regulation of LFA-1 in interaction between lymphocytes and high endothelial cells. *Eur. J. Immunol.*, 21 855 - 858.
2. Tamatani, T., Kotani, M., Miyaska, M., Characterization of the rat leukocyte integrin, CD11/CD18, by the use of LFA-1 subunit-specific monoclonal antibodies. *Eur. J. Immunol.* 21:627-633 (1991)
3. Yamazaki, T. *et al.* Expression of intercellular adhesion molecule-1 in rat heart with ischemia/reperfusion and limitation of infarct size by treatment with antibodies against cell adhesion molecules. *Am. J. of Path.* 143:410-418

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