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# TECHNICALLY *Speaking*

Place your order with CEDARLANE® or your local distributor.  
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## FITC Anti-Rat CD11a (LFA-1 $\alpha$ Chain) Monoclonal Antibody

CL017F  
CL017F-5

LOT: 1731

### DESCRIPTION:

LFA-1 (lymphocyte function associated molecule-1) is one of the leukocyte integrins. It is a heterodimer consisting of  $\alpha$  and  $\beta$  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 CL017F is specific for the  $\alpha$  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 and immunohistochemistry.

### PRESENTATION:

100  $\mu$ g (CL017F) or 500  $\mu$ g (CL017F-5) FITC conjugated Ig buffered in PBS, 0.02% NaN<sub>3</sub> 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 repeated freeze/thaw cycles.

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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  $\alpha$ chain )

Ig Class: Mouse IgG<sub>2a</sub>

Format: FITC conjugated Ig buffered in PBS, 0.2 % NaN<sub>3</sub> and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml.

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<sup>®</sup>-Rat cell separation medium (CL5040).
2. Wash 2 times.
3. Resuspend the cells to a concentration of  $2 \times 10^7$  cells/ml in media A. Add 50  $\mu$ l of this suspension to each tube (each tube will then contain  $1 \times 10^6$  cells, representing 1 test).
4. To each tube, add 1.0  $\mu$ g\* of **CL017F** 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.  
(It is recommended that the tubes are protected from light, since most fluorochromes are light sensitive.)
7. Wash 2 times at 4°C.
8. Resuspend the cell pellet in 50  $\mu$ l ice cold media B.
9. 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  $\mu$ l of 2M sodium azide in 100 mls).
- B. Phosphate buffered saline (pH 7.2) + 0.5 % bovine serum albumin + sodium azide (100  $\mu$ l of 2M sodium azide in 100 mls).

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Results:

Tissue Distribution by Flow Cytometric Analysis:

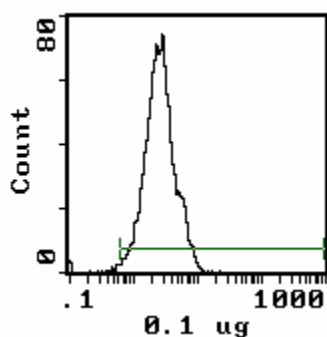
Rat Strain: Wistar

Cell Concentration:  $1 \times 10^6$  cells per test

Antibody Concentration Used:  $0.1 \mu\text{g}/10^6$  cells

Isotypic Control: FITC Mouse IgG<sub>2a</sub> (CLCMG2A01)

<u>Cell Source</u>	<u>Percentage of cells stained above control:</u>
Thymus	83.0%
Spleen	12.8%
Bone Marrow	34.8%



LFL1

**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.

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