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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

TECHNICALLY Speaking

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

Purified Anti-Rat CD11a (LFA-1 a Chain) Monoclonal Antibody

CL017AP
LOT: 03010304

DESCRIPTION:

LFA-1 (lymphocyte function associated molecule-1) is one of the leukocyte integrins. It is a heterodimer consisting of a and b 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 CL017AP is specific for the a 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 (frozen), and functional testing.

PRESENTATION: 200µg, purified Ig fraction in PBS containing 0.1% sodium azide (NaN_3).

STORAGE/STABILITY:

Store at 4°C for short term. For long term storage, aliquot and freeze unused portions at -20°C in volumes appropriate for single usage. Avoid repeated freeze/thaw cycles.

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

CEDARLANE® LABORATORIES LIMITED
5516 - 8th Line, R.R.#2, Hornby, Ontario, CANADA L0P 1E0

toll free: 1-800-268-5058
in North America
phone: (905) 878-8891 • fax: (905) 878-7800

or visit our website for a list of our international distributors including contact information
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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 a chain)

Ig Class: Mouse IgG_{2a}

Format: Purified Ig buffered in PBS and 0.02% NaN_3. (Purified from ascitic fluid via Protein G Chromatography).

Antibody Concentration: 1.0 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 $2x10^7$ cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contains $1x10^6$ cells representing 1 test).
4. To each tube add ~1.0 µg of **CL017AP**.
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 **CLCC30201** (FITC goat anti-mouse IgG (H+L)) 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 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 phosphate buffered saline. (This stains dead cells by intercalating DNA).

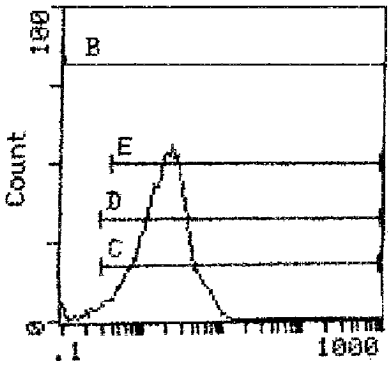
Media:

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:
(Representative Histogram)
Rat Strain: Wistar
Cell Concentration: $1x10^6$ cells per test
Antibody Concentration Used: 1.0 µg/10^6 cells
Isotypic Control: Purified Mouse IgG_{2a},k (CLCMG2A00)

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.**

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5. Tamatani, T., Miyasaka M: Identification of monoclonal antibodies reactive with the rat homolog of ICAM-1, and evidence for a differential involvement of ICAM-1 in the adherence of resting versus activated lymphocytes to high endothelial cells. *International Immunol.* 1990, 2: 165-171.

FOR RESEARCH USE ONLY

NWB 01/06/02