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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 CD25 (IL-2R) Monoclonal Antibody

CL039B

CL039B-5

LOT: 3942

DESCRIPTION:

Cedarlane's anti-rat Interleukin-2 receptor monoclonal antibody recognizes the smaller (alpha subunit) 55kD chain of the IL-2 receptor found on activated rat T cells, thymic dendritic cells but not resting lymphocytes (1). CL039B binds to the rat interleukin-2 receptor and has proven to be an important marker for activated T cells (2).

This clone is reported to work with frozen and paraffin sections (3).

PRESENTATION:

100 µg (CL039B) or 500 µg (CL039B-5) Biotin conjugated Ig buffered in PBS, 0.02% NaN₃ 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 freeze/thaw cycles.

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: MRC OX-39

Hybridoma Production:

Immunization: Immunogen: T blasts from a mixed lymphocyte reaction between purified CD4 positive T cells and irradiated spleens.

Donor: BALB/c spleen

Specificity: Rat CD25 (IL-2R)

Ig Class: Mouse IgG₁

Format: Biotin conjugated Ig buffered in PBS, 0.02% NaN₃ and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml. (Purified from bioreactor supernatant)

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[®]-Rat cell separation medium (CL5040).
2. Wash 2 times.
3. Resuspend the 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 contain 1×10^6 cells, representing 1 test).
4. To each tube, add 1.0-.5ug* of **CL039B or CL039B-5** 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.
7. Wash 2 times at 4°C.
8. Add 100 μ l of secondary antibody **CLCSA1004** (Streptavidin-PE) at a 1:50 dilution.
9. Incubate tubes at 4°C for 30 - 60 minutes (It is recommended that tubes are protected from light since most fluorochromes are light sensitive).
10. Wash 2 times at 4°C.
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 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:

Rat Strain: Fischer

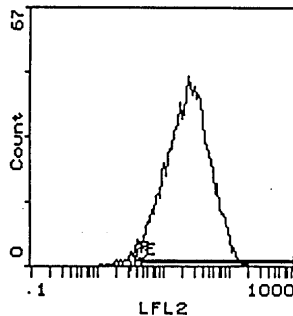
Cell Concentration : 1×10^6 cells per test

Antibody Concentration Used: $0.5 \mu\text{g}/10^6$ cells

Isotypic Control: Biotin Mouse IgG₁ (CLCMG115)

Cell SourcePercentage of cells stained above control:

T Cell Blasts	97.8%
Thymus	0.5%



Cell Source: T Cell Blasts

Percentage of cells stained above control: 97.8%

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) Paterson, D.J., Jeffries, W.A., Green, J.R., Brandon, M.R., Corthesy, P., Puklavec, M. and A.F. Williams. (1987) Mol. Immunol. 24, 1281-1290. Antigen of Activated Rat T Lymphocytes Including a Molecule of 50,000 Mr Detected Only on CD4 Positive T Blasts
- 2) Barclay, A.N. (1981) Immunology. 42 593-600 The Localization of populations of lymphocytes defined with monoclonal antibodies in rat lymphoid tissues.
- 3) Whiteland, J.L et al (1995). Immunohistochemical detection of T cell subsets and other leukocytes in paraffin embedded rat and mouse tissues with monoclonal antibodies .J. Histochem. Cytochem. 43: 313-320.

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