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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 RT1.B Monoclonal Antibody

CL010B
CL010B-5
LOT: 1041

DESCRIPTION:

Cedarlane's biotin anti-rat RT1.B monoclonal antibody recognizes Ia from all rat strains tested. It labels a monomorphic determinant of the rat Ia antigen present on the majority of peripheral B lymphocytes, dendritic cells, some macrophages, and certain epithelial cells (2,3,4) but not peripheral T lymphocytes. (4) This antibody also cross reacts with murine Ia antigens (particularly with k and s haplotypes). Analysis of recombinant strains shows that the determinant maps to the I-A region of the mouse MHC (1,2) and correlates with mouse Ia specificity 17 or 18. Thus, the determinant recognized by CL010B is polymorphic in the mouse. This antibody may be useful for recognizing the rat Ia antigen and can be coupled to Sepharose 4B for purification of rat Ia antigen by antibody affinity chromatography (1).

This clone is reported to work with frozen and paraffin sections (5).

PRESENTATION:

100 µg (CL010B) or 500 µg (CL010B-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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SPECIFICATIONS:

Clone: MRC OX-6

Hybridoma Production:

Immunization: Immunogen: Rat thymocyte membrane glycoprotein
Donor: BALB/c spleen

Fusion Partner: PS-NS1-1-Ag4 (NS1/1)

Specificity: Rat RT1.B (Rat Ia-non-polymorphic)

Ig Class: Mouse IgG₁

Format: Biotin conjugated Ig buffered in PBS, 0.02% NaN₃ and EIA grade BSA as a stabilizing protein concentration to 4-5 mg/ml.. (Purified from ascitic fluid via Protein G Chromatography)

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 0.1 - 0.5 μ g of **CL010B** or **CL010B-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 **CLCSA1001** (Streptavidin-FITC) at a 1:500 dilution.
9. Incubate the 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

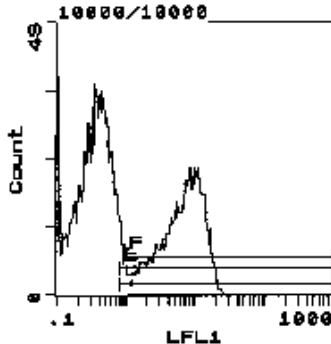
Cell Concentration : 1×10^6 cells per test

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

Isotypic Control: Biotin Mouse IgG₁, κ (CLCMG115)

Cell SourcePercentage of cells stained above control:

Thymus	26.1%
Spleen	39.0%
Lymph Node	17.0%



Cell Source: Spleen

Percentage of cells stained above control: 39.0%

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

Cell Concentration: 1×10^6 cells per test

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

Strains Tested: Fischer, Wistar, Buffalo

Positive: Fischer, Wistar, Buffalo

Negative: none

REFERENCES:

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- 3) Barclay, A.N. and G. Mayrhofer. (1981) Bone Marrow origin of Ia-positive cells in the medulla of rat thymus. *J. Exp. Med.* 153, 1666-1671.
- 4) Barclay, A.N. (1981) Different reticular elements in rat lymphoid tissue identified by localization of Ia, Thy-1 and MRC OX-2 antigens. *Immunology.* 44, 727-736.
- 5) Fukumoto, T et al (1982) Mouse monoclonal antibodies against rat major histocompatibility antigens. Two Ia antigens and expression of Ia and class I antigens in rat thymus. *Eur. J. Immunol.* 12:237-243.
- 6) 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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