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TECHNICALLY Speaking

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

Anti-Rat RT1.B
Monoclonal Antibody-Ascites

CL010A
LOT: 1014

DESCRIPTION:

Cedarlane's 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 CL010A 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: 0.5 ml, lyophilized ascites.

STORAGE/STABILITY:

Lyophilized form stable at 4°C or -20°C. Reconstitute with 0.5 ml of cold distilled water. After reconstitution, aliquot and freeze unused portions in volumes appropriate for single usage. Avoid repeated freeze/thaw cycles.

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

CEDARLANE® LABORATORIES LIMITED

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SPECIFICATIONS:

Clone: MRC OX-6

Hybridoma Production:

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

Fusion Partner: myeloma cell line P3-NSI-1-Ag4 (NS1/1)

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

Ig Class: Mouse IgG_1

Format: Ascitic fluid filtered to 0.45 µ (non-sterile) and lyophilized

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 $2x10^7$ cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain $1x10^6$ cells, representing 1 test).
4. To each tube, add 50 µl of a 1:10,000-1:20,000 dilution ***** of **CL010A**.
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 1:500 dilution.
9. Incubate the 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 PBS. This stains dead cells by intercalating in DNA.

Media:

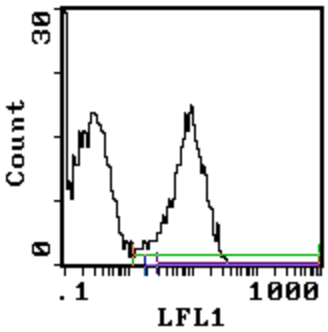
A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 µl of 2M sodium azide in 100 mls).
B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 µl of 2M sodium azide in 100 mls).

Results:

Tissue Distribution by Flow Cytometry Analysis:

Rat Strain: Fischer
Cell Concentration : $1x10^6$ cells per tests
Antibody Concentration Used: 1:10,000 in 50 µl /10^6 cells
Isotypic Control: Mouse IgG_1,κ

Cell Source	Percentage of cells stained above control:
Thymus	10.6%
Spleen	41.0%
Lymph Node	24.2%



Cell Source: Spleen
Percentage of cells stained above control: 41.0 %

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.**

REFERENCES:

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2. Barclay, A.N. (1981) The localization of populations of lymphocytes defined by monoclonal antibodies in rat lymphoid tissues. Immunology. 42, 593-600..
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. 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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