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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.D Monoclonal Antibody

CL011B
CL011B-5
LOT: 1142

DESCRIPTION:

Cedarlane's anti-rat RT1.D monoclonal antibody recognizes a monomorphic determinant on the α chain of the rat Ia antigen and appears to be the rat homologue of mouse Ia-E. It recognizes the rat Ia product present on B, but not T cells from lymph node or thoracic duct lymph. It does not bind to thymocytes or erythrocytes. The antibody does not cross-react with rat Ia-A or mouse Ia-E antigen, but rabbit antibody raised against the antibody affinity column-purified MRC OX-17 antigen cross-reacted on tissues of mice expressing Ia-E mouse antigen but not on those mouse strains that were Ia-E antigen negative (2).

PRESENTATION:

100 µg (CL011B) or 500 µg (CL011B-5) Biotin conjugated Ig buffered in PBS, 0.02% NaN_3 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-17

Hybridoma Production:

Immunization: Immunogen: Rat spleen membrane glycoproteins depleted of Ia-A antigens.
Immunocyte Donor: BALB/c spleen
Fusion Partner: X63 Ag8.653

Specificity: Rat RT1.D (Rat Ia-E)

Ig Class: Mouse IgG_1

Format: Biotin conjugated Ig buffered in PBS, 0.02% NaN_3 and EIA grade BSA as a stabilizing protein to bring total 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 $2x10^7$ cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1×10^6 cells, representing 1 test).
4. To each tube, add 0.5-0.1 µg***** of **CL011B or CL011B-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 µl of secondary antibody **CLCSA1001** (Streptavidin-FITC) at a 1:500 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 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:

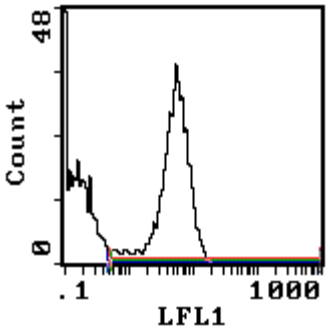
A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 µl of 2M sodium azide in 100 mls).
B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 µl of 2M sodium azide in 100 mls).

Results:

Tissue Distribution by Flow Cytometry Analysis:

Rat Strain: Fischer
Cell Concentration : $1x10^6$ cells per test
Antibody Concentration Used: 0.1 µg/10^6 cells
Isotypic Control: Biotin Mouse IgG_1

Cell Source	Percentage of cells stained above control:
Thymus	6.1%
Spleen	48.8%
Lymph Node	27.5%



Cell Source: Spleen
Percentage of cells stained above control: 48.8%

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. Kearney, J.F., Radbruch, A., Leisegang, B. and K. Rajewsky. (1979) A New mouse myeloma cell line that has lost Immunoglobulin expression but permits the construction of antibody-secreting hybrid cell lines. J. Immunol. 123, 1548-1550.

2. Fukumoto, T., McMaster, W.R. and A.F. Williams. (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.

3. Barclay, A.N. (1981) Different reticular elements in rat lymphoid tissue identified by localization of Ia, thy-1 and MRC OX-2 antigens. Immunology. 42, 593-600.

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