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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^u Monoclonal Antibody

CL008B

CL008B-5

LOT: 0841

DESCRIPTION:

Cedarlane's anti-rat RT1.B^u monoclonal antibody recognizes a polymorphic determinant on rat Ia present on Lewis, Wistar, and AO rat strains (although it is apparently expressed in lower amounts in AO rats) but not BN, DA, or PVG/c strains (1,2). This antibody also cross-reacts with mouse strains (MHC haplotypes b and s) and analysis of recombinant mouse strains shows that the determinant maps to the I-A region and correlates with mouse Ia specificity 9 (1). CL008B recognizes Ia antigens on approximately 18% of thymocytes but does not significantly label the majority of peripheral T lymphocytes (1,3,4). Thus, CL008B is particularly useful for distinguishing Ia positive cells from different rat strains and has been used in the recognition of cells of donor origin in bone marrow reconstituted radiation chimeras (3,4).

PRESENTATION:

100 µg (CL008B) or 500 µg (CL008B-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-3

Hybridoma Production:

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

Fusion Partner: P3-NS1-1-Ag4 (NS1/1)

Specificity: Rat RT1.B^U (Rat Ia-polymorphic)

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 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 0.05 μ g* of **CL008B or CL008B-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 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: Buffalo

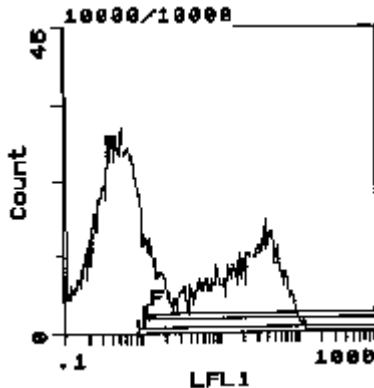
Cell Concentration : 1×10^6 cells per test

Antibody Concentration Used: $0.05 \mu\text{g}/10^6$ cells

Isotypic Control: Biotin Mouse IgG₁

Cell SourcePercentage of cells stained above control:

Thymus	25.8%
Spleen	44.3%
Lymph Node	10.0%



Cell Source: Spleen

Percentage of cells stained above control: 44.3 %

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.

Strain Distribution by Flow Cytometry Analysis:

Procedure: see page 2

Cell Concentration: 1×10^6 cells per test

Antibody Concentration Used: $0.05 \mu\text{g}/10^6$ cells

Strains Tested: Wistar, Buffalo, Brown Norway, ACI, Fischer 344,
Lewis

Positive: Wistar, Buffalo, Fischer 344, Lewis

Negative: Brown Norway, ACI

REFERENCES:

1. McMaster, W.R. and A.F. Williams. (1979) Eur. J. Immunol. 9, 426-433. Identification of Ia glycoproteins in rat thymus and purification from rat spleen.
2. McMaster, W.R. and A.F. Williams. (1979) Immunol. Rev. 47, 117-137. Monoclonal Antibodies to Ia antigens from Rat Thymus: Cross Reactions with mouse and human and use in purification of rat IA glycoproteins.
3. Barclay, A.N. and G. Mayrhofer. (1981) J. Exp. Med. 153, 1666-1671. Bone Marrow origin of Ia-positive cells in the medulla of rat thymus.
4. Barclay, A.N. (1981) Immunology. 44, 727-736. Different reticular elements in rat lymphoid tissue identified by localization of Ia, Thy 1 and MRC OX-2 antigens.

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