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TECHNICALLY Speaking

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

FITC Anti-Rat RT1.B^{u} Monoclonal Antibody

CL008F
CL008F-5
LOT: 0831

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). CL008F recognizes Ia antigens on approximately 18% of thymocytes but does not significantly label the majority of peripheral T lymphocytes (1,3,4). Thus, CL008F 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 (CL008F) or 500 µg (CL008F-5) FITC 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...

CEDARLANE® LABORATORIES LIMITED

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in North America

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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 IgG_1

Format: FITC 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 0.02 µg***** of **CL008F or CL008F-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.
   (It is recommended that the tubes are protected from light, since most fluorochromes are light sensitive.)
7. Wash 2 times at 4°C.
8. Resuspend the cell pellet in 50 µl ice cold media B.
9. 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:

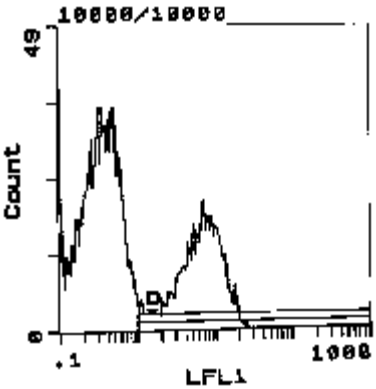
A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 µl of 2M sodium azide in 100 mls).
B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 µl of 2M sodium azide in 100 mls).

Results:

Tissue Distribution by Flow Cytometry Analysis:

Rat Strain: Buffalo
Cell Concentration : $1x10^6$ cells per test
Antibody Concentration Used: 0.02 µg/10^6 cells
Isotypic Control: FITC Mouse IgG_1

Cell Source	Percentage of cells stained above control:
Thymus	21.8%
Spleen	30.3%
Lymph Node	15.1%



Cell Source: Spleen
Percentage of cells stained above control: 30.3%

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 x 106 cells per test
Antibody Concentration Used: 0.05 µg/106 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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