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TECHNICALLY *Speaking*

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

PE Anti-Rat RT1.B^u Monoclonal Antibody

CL008PE
CL008PE-4
LOT: 0851

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, ACI, 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). CL008PE recognizes Ia antigens on approximately 18% of thymocytes but does not significantly label the majority of peripheral T lymphocytes (1,3,4). Thus, CL008PE 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:

50 µg (CL008PE) or 200 µg (CL008PE-4) R-PE 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. **DO NOT FREEZE.** Avoid prolonged exposure to light.

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

CEDARLANE®
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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: R-PE 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 **CL008PE or CL008PE-4** 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 fluorochemicals are light sensitive.)
7. Wash 2 times at 4°C.
8. Resuspend the cell pellet in 50 μ l ice cold media B.
9. 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

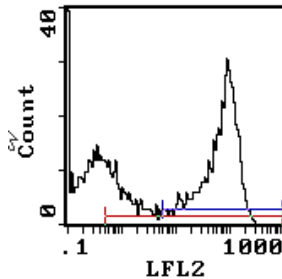
Cell Concentration : 1×10^6 cells per tests

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

Isotypic Control: PE Mouse IgG₁

Cell SourcePercentage of cells stained above control:

Thymus	18.6%
Spleen	43.9%
Lymph Node	20.8%



Cell Source: Spleen

Percentage of cells stained above control: 43.9%

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.**

R-Phycoerythrin conjugates are produced under license and protected under Stanford University held patents 4,520,110; 4,542,104; 4,859,582; 5,055,556 (U.S.); 76695 (EPC); 548440 (Australia); 1,179,942 (Canada); and 1,594,827 (Japan).

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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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