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Monoclonal Antibody to MHC Class II (RT1D) - FITC

Catalog No.: CL121FX

Quantity: 0.5 mg

Concentration: 0.1 mg/ml

Host / Isotype: Mouse / IgG1

Clone: OX-17

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

Format: **State:** Liquid purified Ig
Purification: Protein G Chromatography
Buffer System: PBS, 0.02% NaN3 and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml
Label: FITC

Applications: Flow Cytometry.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This 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.
Species: Rat.
Other species not tested.

Storage: Store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer.
Avoid repeated freezing and thawing.
This antibody is photosensitive and should protected from light.
Shelf life: one year from despatch.

General 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.

Protocols:

FLOW CYTOMETRY ANALYSIS:

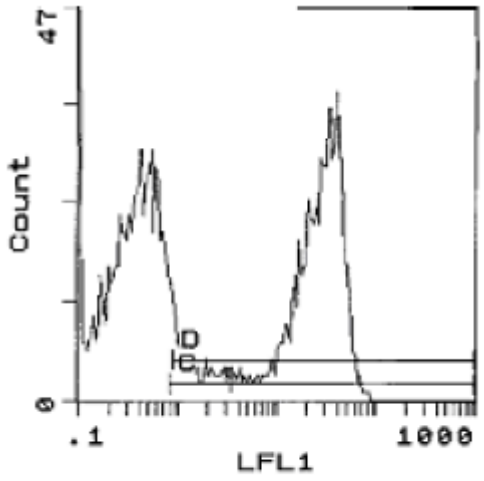
Method:
1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population with Lympholyte®-Rat Cell Separation Medium.
2. Wash 2 times.
3. Resuspend the cells to a concentration of 2x10e7 cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1 x 10e6 cells, representing 1 test).
4. To each tube, add 0.05-0.1 µg* of this Ab per 10e6 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:
Rat Strain: Fischer
Cell Concentration: 1x10e6 cells per test
Antibody Concentration Used: 0.1 µg/10e6 cells
Isotypic Control: FITC Mouse IgG1

Cell Source Percentage of cells stained above control:
Thymus 10.2%
Spleen 49.0%
Lymph Node 27.9%

Pictures:



Cell Source: Spleen
Percentage of cells stained above control: 49.0%