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Monoclonal Antibody to MHC Class II I-Ad - FITC

Catalog No.: CL090F

Quantity: 0.1 mg

Concentration: 0.1 mg/ml

Host / Isotype: Mouse / IgG2a

Clone: 34-5-3S

Immunogen: BDF spleen
Donor: C3H/He spleen
Fusion Partner: SP2/0-Ag14

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 cytotoxic monoclonal antibody specific for cells expressing the Ia antigen coded for by the A subregion of the d, b, p, and q haplotypes. (ie. I-Ad,b,p,q).
Species: Mouse.
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.
Shelf life: one year from despatch.

General References:
1. Ozato, K. et al. 1982. Monoclonal Antibodies to Mouse Major Histocompatibility Complex Antigens. Transplantation. 34: 113-120.
2. Ahn, H.J. et al. 1997. A Mechanism Underlying Synergy Between IL-12 and IFN-g-Inducing Factor in Enhanced Production of IFN-g. Journal of Immunology. 159: 2125-2131.

Protocols:

FLOW CYTOMETRY ANALYSIS:

Method:
1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population with Lympholyte®-M 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 0.2 - 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.

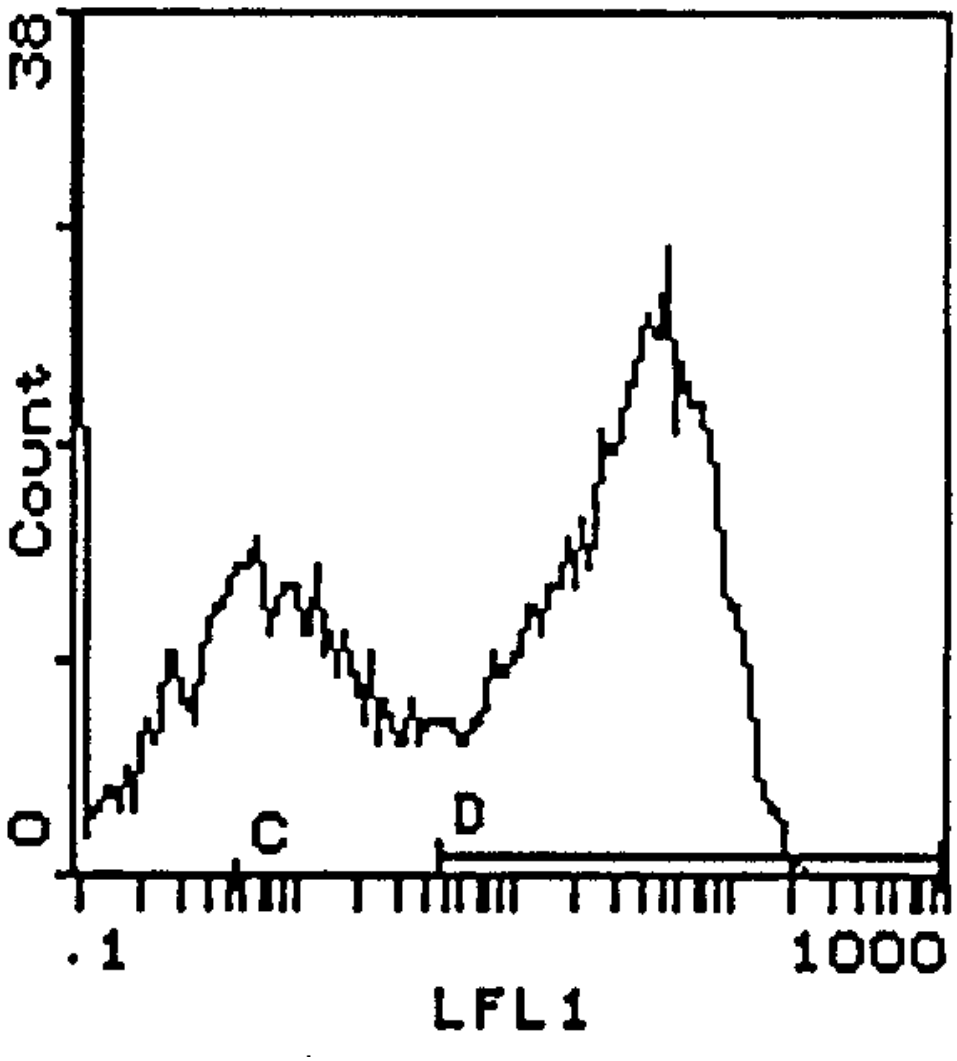
Media:
A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 µl of 2M sodium azide in 100 mls).
B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 µl of 2M sodium azide in 100 mls).

Results - Tissue Distribution by Flow Cytometry Analysis:
Mouse Strain: BALB/c
Cell Concentration : 1x10e6 cells per test
Antibody Concentration Used: 0.1 µg/10e6 cells
Isotypic Control: FITC Mouse IgG2a

Cell Source - Percentage of cells stained above control:
Spleen: 58.7%
Lymph Node: 23.4%
Thymus: 53.9%

Strain Distribution by Flow Cytometry Analysis:
Antibody Concentration: 0.2 µg/10e6 cells
Strains Tested: A.TH, A.TL, C3H/He, C57BL/6, DBA/1
Positive: C57BL/6, DBA/1
Negative: A.TH, A.TL, C3H/He

Pictures:



Cell Source: Spleen - Percentage of cells stained above control: 58.7%