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Monoclonal Antibody to MHC Class II I-Ap - FITC

Catalog No.: CL072F

Quantity: 0.1 mg

Concentration: 0.1 mg/ml

Host / Isotype: Mouse / IgG2a

Clone: 7-16.17

Immunogen: B10.p
Donor: BALB/c
Fusion Partner: SP2/0

Format: **State:** Liquid purified
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 is a cytotoxic antibody which defines a public I-A antigen. This antibody reacts with I-A antigen from the following I-A haplotypes: I-Ap,k,q,r,s,b. Using recombinant strains, reactivity against the b haplotype has been localized to the Ab subregion. This antibody can be used to quantitate or eliminate I-A bearing cells for precipitating I-A antigen.
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.
This product is photosensitive and should protected from light.
Shelf life: one year from despatch.

General References:
1. Harmon, R.C., Stein, N., Frelinger, J.A. 1983. Immunogenetics 18:541-545.

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 0.1 - 0.2 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: BDP
Cell Concentration: 1x10e6 cells per tests
Antibody Concentration Used: 0.1 µg/10e6 cells
Isotypic Control: FITC Mouse IgG2a

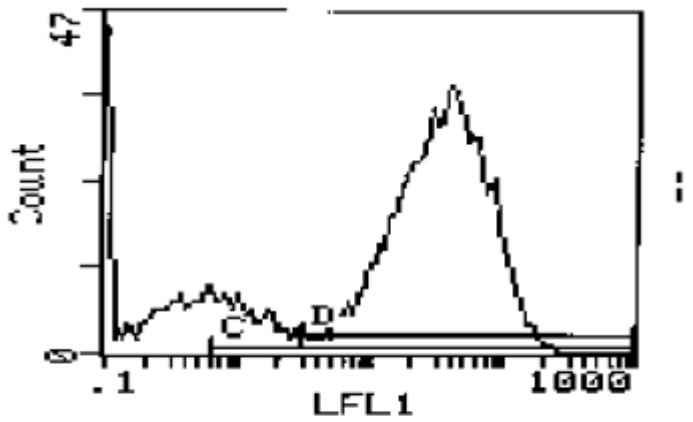
Cell Source - Percentage of cells stained above control:
Spleen: 76.7%
Lymph Node: 40.5%
Bone Marrow: 39.4%
Thymus: 55.6%

Strain Distribution by Flow Cytometry Analysis:
Procedure: As above
Antibody Concentration: 0.2 µg/10e6 cells
Strains Tested: see **FIGURE 2** For a more detailed strain distribution - see reference 1.

Pictures:

Strain	H-2 Loci Alleles								+/-
	K	A_β	A_α	E_β	E_α	C4	C4S	D	
BDP	s	s	s	s	s	s	s	d	+
A.TH	s	s	s	s	s	s	s	d	+
C3H/He	k	k	k	k	k	k	k	k	+
C57BL/6	b	b	b	b	b	b	b	b	+
BALB/c	d	d	d	d	d	d	d	d	-

Figure 2: strain distribution



Cell Source: Spleen
Percentage of cells stained above control: 76.7%

Figure 1