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Monoclonal Antibody to MHC Class II (I-Ek) - PE

Alternate names: MHC class II antigen I-Ek

Catalog No.: CL074R

Quantity: 50 µg

Concentration: 0.1 mg/ml

Host / Isotype: Mouse / IgG2a

Clone: 14-4-4S

Immunogen: C3H
Donor: C3H.SW
Fusion Partner: SP2/0-Ag 14

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: PE

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 specific for cells expressing the Ia antigen coded for by the Ea subregion. The reaction pattern of this antibody with a panel of standard and recombinant haplotypes demonstrates that this antibody reacts with antigen Ia.m7, which is expressed by all haplotypes except b,f,q and s. This antibody can be used to quantitate or eliminate cells bearing the Ia.m7 antigen and is well suited for identifying Ia cell populations of positive mouse strains.
Species: Mouse.
Other species not tested.

Storage: Store the antibody undiluted at 2-8°C.
DO NOT FREEZE!
This product is photosensitive and should protected from light.
Shelf life: one year from despatch.

General References:
1. Ozato, K., Mayer, N., and Sachs, D.H. 1980. Hybridoma cell line secreting monoclonal antibodies to mouse H-2 and Ia Antigens. J. Immunol.: 214:533-540.

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 0.5 - 1.0 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 flurochromes 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: CBA/J
Cell Concentration: 1x10e6 cells per tests
Antibody Concentration Used: 0.5 µg/10e6 cells
Isotypic Control: PE Mouse IgG2a

Cell Source Percentage of cells stained above control:
Spleen: 62.2% see **FIGURE 1**
Lymph Node: 12.5%
Bone Marrow: 39.0%

Strain Distribution by Flow Cytometry Analysis:
Procedure: As above
Antibody Concentration: 0.5 µg/106 cells
Strains Tested: see **FIGURE 2;** For a more detailed strain distribution - see reference 1.

Pictures:

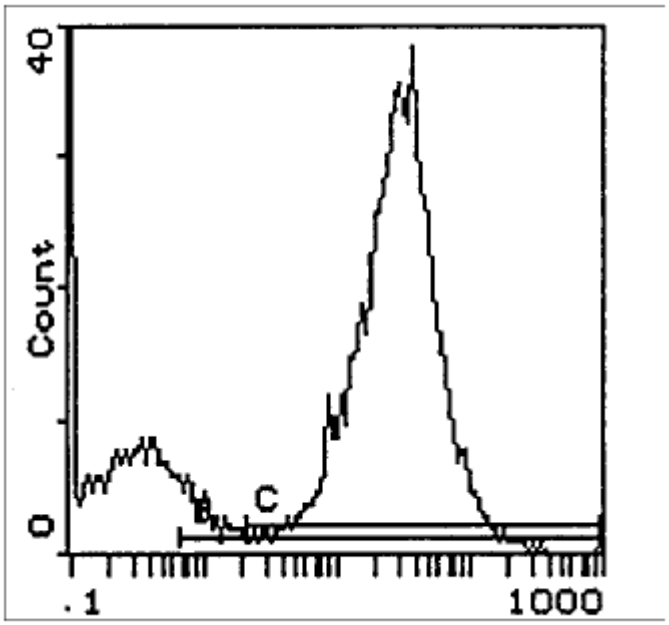

Figure 1

Strain	H-2 Loci Alleles								+/-
	K	A_β	A_α	E_β	E_α	C4	C4S	D	
A.TH	s	s	s	s	s	s	s	d	-
B10.A(3R)	b	b	b	b/k	k	d	d	d	+
AKR	k	k	k	k	k	k	k	k	+
C3H/He	k	k	k	k	k	k	k	k	+
A.TL	s	k	k	k	k	k	k	d	+
C57BL/6	b	b	b	b	b	b	b	b	-
BALB/c	d	d	d	d	d	d	d	d	+

Figure 2