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Product datasheet for CL072B

MHC Class II I-Ap Mouse Monoclonal Antibody [Clone ID: 7-16.17]

Product data:

Product Type:	Primary Antibodies
Clone Name:	7-16.17
Applications:	FC
Recommended Dilution:	Flow Cytometry.
Reactivity:	Mouse
Host:	Mouse
Isotype:	IgG2a
Clonality:	Monoclonal
Immunogen:	B10.P Donor: BALB/c Fusion Partner: SP2/0
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 or for precipitating I-A antigen.
Formulation:	PBS, 0.02% NaN3 and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml. Label: Biotin State: Liquid purified Ig
Concentration:	lot specific
Purification:	Protein G Chromatography
Conjugation:	Biotin
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.
Stability:	Shelf life: one year from despatch.

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Note:

Protocol: **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 1.0 - 0.5 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.
7. Wash 2 times at 4°C.
8. Add 100 µl of secondary antibody (Streptavidin-FITC) at a 1:500 dilution.
9. Incubate tubes at 4°C for 30 - 60 minutes (It is recommended that tubes are protected from light since most fluorochromes are light sensitive).
10. Wash 2 times at 4°C.
11. Resuspend the cell pellet in 50 µl ice cold media B.
12. 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: 0.5 µg/10e6 cells
Isotypic Control: Biotin Mouse IgG2a

Cell Source Percentage of cells stained above control:
Thymus: 36.5%
Spleen: 51.5%
Lymph Node: 16.3%
Bone Marrow: 24.4%

Strain Distribution by Flow Cytometry Analysis:
Antibody Concentration Used: 0.5 µg/10e6 cells
Strains Tested: see **FIGURE 2**; For a more detailed strain distribution - see reference 1.

Product images:

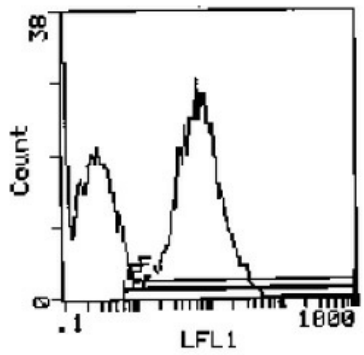

Figure 1

Cell Source: Spleen
Percentage of cells stained above control: 51.5%

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