

## Product datasheet for **CL072F**

### 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 for precipitating I-A antigen.
Formulation:	PBS, 0.02% NaN <sub>3</sub> and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml. Label: FITC State: Liquid purified
Concentration:	lot specific
Purification:	Protein G Chromatography
Conjugation:	FITC
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 be protected from light.
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  $2 \times 10^7$  cells/ml in media A. Add 50  $\mu$ l of this suspension to each tube (each tube will then contain  $1 \times 10^6$  cells, representing 1 test).
4. To each tube, add 0.1 - 0.2  $\mu$ g\* of this Ab per  $10^6$  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  $\mu$ l ice cold media B.
9. Transfer to suitable tubes for flow cytometric analysis containing 15  $\mu$ l 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  $\mu$ l of 2M sodium azide in 100 mls).
- B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100  $\mu$ l of 2M sodium azide in 100 mls).

**Results - Tissue Distribution by Flow Cytometry Analysis:**

Mouse Strain: BDP

Cell Concentration:  $1 \times 10^6$  cells per tests

Antibody Concentration Used: 0.1  $\mu$ g/ $10^6$  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  $\mu$ g/ $10^6$  cells

Strains Tested: see **FIGURE 2** For a more detailed strain distribution - see reference 1.

Product images:

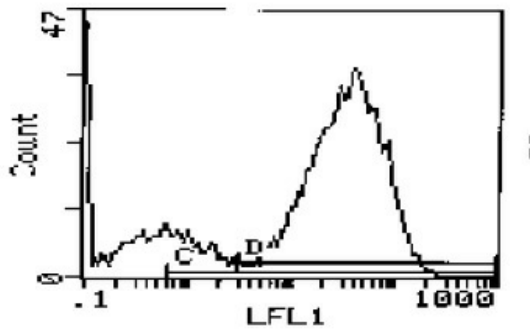


Figure 1

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

Strain	H-2 Loci Alleles	+/-
	<u>K</u> <u>A<sub>β</sub></u> <u>A<sub>α</sub></u> <u>E<sub>β</sub></u> <u>E<sub>α</sub></u> <u>C4</u> <u>C4S</u> <u>D</u>	
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