

## Product datasheet for **CL074R**

### MHC Class II I-Ek Mouse Monoclonal Antibody [Clone ID: 14-4-4S]

#### Product data:

Product Type:	Primary Antibodies
Clone Name:	14-4-4S
Applications:	FC
Recommended Dilution:	Flow Cytometry.
Reactivity:	Mouse
Host:	Mouse
Isotype:	IgG2a
Clonality:	Monoclonal
Immunogen:	C3H Donor: C3H.SW Fusion Partner: SP2/0-Ag 14
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.
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: PE State: Liquid purified Ig
Concentration:	lot specific
Purification:	Protein G Chromatography
Conjugation:	PE
Storage:	Store the antibody undiluted at 2-8°C. DO NOT FREEZE! 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 0.5 - 1.0  $\mu$ 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 fluorochemicals 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: CBA/J

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

Antibody Concentration Used: 0.5  $\mu$ g/ $10^6$  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  $\mu$ g/ $10^6$  cells

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

Product images:

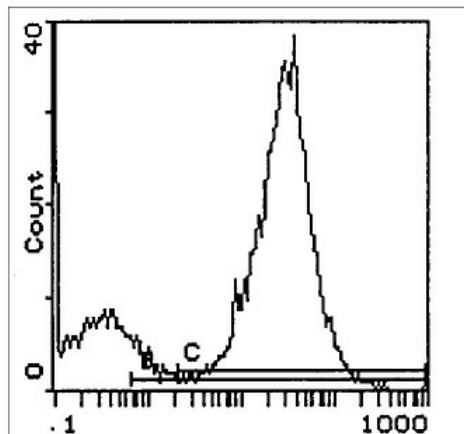


Figure 1

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>	
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