OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850, US
Phone: +1-888-267-4436
https://www.origene.com
techsupport@origene.com
EU: info-de@origene.com
CN: techsupport@origene.cn

Product datasheet for CL074B

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 is well suited for the quantitation and elimination of Ia bearing cells.
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 0.2-0.5 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: CBA/J
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:
Spleen: 53.4% see **FIGURE 1**
Lymph Node: 16.5%
Bone Marrow: 25.6%

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:

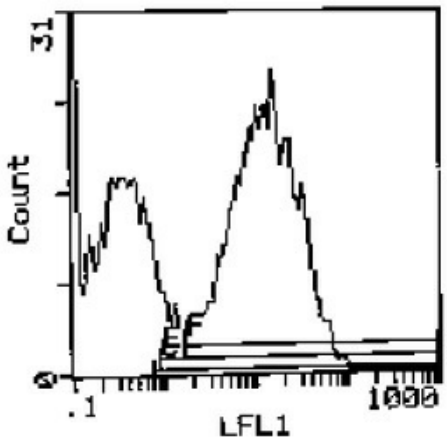

Figure 1

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