

AM31877RP-N**Monoclonal Antibody to MHC Class II (I-Ab,d) - PE**

Quantity:	50 µg
Concentration:	0.1 mg/ml
Host / Isotype:	Mouse / IgG2a
Clone:	25-9-17S
Immunogen:	C3H.SW splenocytes. Donor: C3H lymphoid cells. Fusion Partner: Sp2/O-Ag14
Format:	State: Liquid purified Ig fraction Purification: Protein G Chromatography Buffer System: PBS containing 0.02% Sodium Azide as preservative and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml Label: PE – R-Phycoerythrin
Applications:	Flow Cytometry (See Protocols). Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.
Specificity:	This Monoclonal Antibody reacts with I-Ab and I-Ad antigens. Cross reaction with H-2p and H-2q was also found. Species: Mouse. Other species not tested.
Storage:	Store the antibody undiluted at 2-8°C. DO NOT FREEZE! This product is photosensitive and should be protected from light. Avoid repeated freezing and thawing. Shelf life: one year from despatch.
General Readings:	1. Ozato, K. and Sachs, D.H. (1981) Journal of Immunology.126, 317-321. Hybridoma antibodies reacting to antigens of the H-2b haplotype reveal genetic control of isotype expression.
Protocols:	<u>FLOW CYTOMETRY ANALYSIS:</u> 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 2x10 ⁷ cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1 x 10 ⁶ cells, representing 1 test). 4. To each tube, add 0.5 µg* of AM31877RP-N 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 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: C57BL/6
 Cell Concentration : 1x10e6 cells per test.
 Antibody Concentration Used: 0.5 µg/106 cells.
 Isotypic Control: PE Mouse IgG2a

Cell Source Percentage of cells stained above control:

Thymus: 20.2%
 Spleen: 60.2%
 Lymph Node: 34.5%

Strain Distribution by Flow Cytometry Analysis:

Antibody Concentration Used: 0.5 µg /10e6 cells
 Strain Testd: See Figure 2.

Pictures:

Figure 2.

Strain	H-2 Loci Alleles					+/-	
	K	A _u A _v	E _u E _v	C4	C4S D		
C57BL/6	b	b	b	b	b	b	+
C3H/He	k	k	k	k	k	k	-
BALB/c	d	d	d	d	d	d	+
DBA/1	q	q	q	q	q	q	(+/-)
SJL	s	s	s	s	s	s	-
B10.M	f	f	f	f	f	f	-
A.TH	s	s	s	s	s	d	-
A.TL	s	k	k	k	k	d	-
B10.A(3R)	b	b	b/k	k	d	d	+
P/J	p	p	p	p	p	p	(+/-)

Figure 1. Cell Source: Spleen. Percentage of cells stained above control:60.2%

