

OriGene Technologies, Inc.

9620 Medical Center Drive, Ste 200 Rockville, MD 20850 UNITED STATES Phone: +1-888-267-4436 Fax: +1-301-340-8606 techsupport@origene.com

## **OriGene Technologies GmbH**

Schillerstr. 5 32052 Herford GERMANY Phone: +49-5221-34606-0 Fax: +49-5221-34606-11 info-de@origene.com

## 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: Sp2/0-Ag14
Format:	State: Liquid purified lg fraction Purification: Protein G Chromatography Buffer System: PBS containing 0.02% Sodium Azideas preservative and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml Label: PE – R-Phycoerythrin
Applications:	<b>Flow Cytometry</b> (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. <b>Species:</b> Mouse. Other species not tested.
Storage:	Store the antibody undiluted at 2-8°C. <b>DO NOT FREEZE!</b> 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:	<ul> <li>FLOW CYTOMETRY ANALYSIS: Method:</li> <li>1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population with Lympholyte®-M cell separation medium.</li> <li>2. Wash 2 times.</li> <li>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 106 cells, representing 1 test).</li> <li>4. To each tube, add 0.5 µg* of AM31877RP-N per 10e6 cells.</li> <li>5. Vortex the tubes to ensure thorough mixing of antibody and cells.</li> <li>6. Incubate the tubes for 30 minutes at 4°C.</li> <li>(It is recommended that the tubes are protected from light, since most fluorochromes are light sensitive.)</li> </ul>

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8. Resuspend the cell pellet in 50  $\mu$ l ice cold media B.

7. Wash 2 times at 4°C.

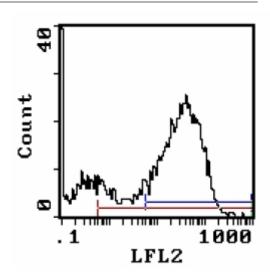
9. Transfer to suitable tubes for flow cytometric analysis containing 15 µl of propidium iodide at 0.5 mg/ml in PBS. This stains dead cells by intercalating in DNA. Media: A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100  $\mu$ l of 2M sodium azide in 100 mls). 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: C57BL/6 Cell Concentration : 1x10e6 cells per test. Antibody Concentration Used:  $0.5 \mu g/106$  cells. Isotypic Control: PE Mouse IgG2a <u>Cell Source Percentage of cells stained above control:</u> 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. H-2 Loci Alleles **Pictures:** Figure 2. Strain

Suam	H-2 LOCI Alleles	
	$\underline{\mathbf{K}} \underline{\mathbf{A}}_{\Box} \underline{\mathbf{A}}_{\Box} \underline{\mathbf{E}}_{\Box} \underline{\mathbf{E}}_{\Box} \underline{\mathbf{C4}} \underline{\mathbf{C4S}} \underline{\mathbf{D}}$	
C57BL/6	b b b b b b b	+
C3H/He	k	-
BALB/c	ddddd d	+
DBA/1	9 9 9 9 9 9 9 9	(+/-)
SJL	5 5 5 5 5 5 5 5	-
B10.M	fffffff	-
A.TH	ssss ss d	-
A.TL	skkkkkd	-
B10.A(3R)	bbb/kkddd	+
P/J	рррррррр	(+/-)

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



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