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

AM31877BT-N Monoclonal Antibody to MHC Class II (I-Ab,d) - Biotin

Quantity: 0.1 mg

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 Ig 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: Biotin

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 for one month or (in aliquots) at -20°C for longer.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General Readings: 1. Ozato K, Sachs DH. Monoclonal antibodies to mouse MHC antigens. III. Hybridoma antibodies reacting to antigens of the H-2b haplotype reveal genetic control of isotype expression. J Immunol. 1981 Jan;126(1):317-21. PubMed PMID: 6935293.

Protocols: **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 106 cells, representing 1 test).
4. To each tube, add 0.5-0.2 µg* of AM31877BT-N 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: C57BL/6
Cell Concentration : 1x10e6 cells per test.
Antibody Concentration Used: 0.5 µg/106 cells.
Isotypic Control: Biotin Mouse IgG2a

Cell Source Percentage of cells stained above control:
Thymus: 17.5%
Spleen: 58.6%
Lymph Node: 31.2%

Strain Distribution by Flow Cytometry Analysis:
Antibody Concentration: 0.5 µg/106 cells.
Strains Tested: See Figure 2.

Pictures:

Figure 2.

Strain	H-2 Loci Alleles								+/-
	K	A_β	A_α	E_β	E_α	C4	C4S	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	+
DBA/1	q	q	q	q	q	q	q	q	(+/-)
SJL	s	s	s	s	s	s	s	s	-
B10.M	f	f	f	f	f	f	f	f	-
A.TH	s	s	s	s	s	s	s	d	-
A.TL	s	k	k	k	k	k	k	d	-
B10.A(3R)	b	b	b	b/k	k	d	d	d	+
P/J	p	p	p	p	p	p	p	p	+

Figure 1. Cell Source: Spleen. Percentage of cells stained above control: 58.6%