

Product datasheet for **AM31877BT-N**

MHC Class II (I-Ab,d) Mouse Monoclonal Antibody [Clone ID: 25-9-17S]

Product data:

Product Type:	Primary Antibodies
Clone Name:	25-9-17S
Applications:	FC
Recommended Dilution:	Flow Cytometry (See Protocols).
Reactivity:	Human
Host:	Mouse
Isotype:	IgG2a
Clonality:	Monoclonal
Immunogen:	C3H.SW splenocytes. Donor: C3H lymphoid cells. Fusion Partner: Sp2/0-Ag14
Specificity:	This Monoclonal Antibody reacts with I-Ab and I-Ad antigens. Cross reaction with H-2p and H-2q was also found.
Formulation:	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: Biotin State: Liquid purified Ig fraction
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 2×10^7 cells/ml in media A. Add 50 μ l of this suspension to each tube (each tube will then contain 1×10^6 cells, representing 1 test).
4. To each tube, add 0.5-0.2 μ g* of AM31877BT-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.
7. Wash 2 times at 4°C.
8. Add 100 μ l 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 μ l ice cold media B.
12. Transfer to suitable tubes for flow cytometric analysis containing 15 μ 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 μ l of 2M sodium azide in 100 mls).

B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 μ l of 2M sodium azide in 100 mls).

Results:

Tissue Distribution by Flow Cytometry Analysis:

Mouse Strain: C57BL/6

Cell Concentration : 1×10^6 cells per test.

Antibody Concentration Used: 0.5 μ g/ 10^6 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 μ g/ 10^6 cells.

Strains Tested: See Figure 2.

Product images:

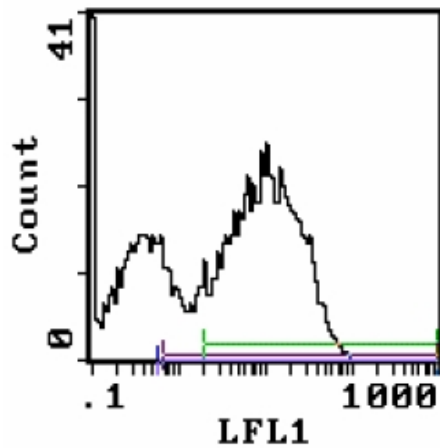


Figure 1. Cell Source: Spleen. Percentage of cells stained above control: 58.6%

Strain	H-2 Loci Alleles	+/-
	<u>K</u> <u>A₁</u> <u>A₂</u> <u>E₁</u> <u>E₂</u> <u>C4</u> <u>C4S</u> <u>D</u>	
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 2.