

## Product datasheet for **AM31877FC-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:	<b>Flow Cytometry</b> (See Protocols).
Reactivity:	Mouse
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: FITC State: Liquid purified Ig fraction Label: Fluorescein
Concentration:	lot specific
Purification:	Protein G Chromatography
Conjugation:	FITC
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. This product is photosensitive and should be protected from light.
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  $2 \times 10^7$  cells/ml in media A. Add 50  $\mu$ l of this suspension to each tube (each tube will then contain  $1 \times 10^6$  cells, representing 1 test).
4. To each tube, add 1.0-0.5  $\mu$ g\* of AM31877FC-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  $\mu$ l ice cold media B.
9. Transfer to suitable tubes for flow cytometric analysis containing 15  $\mu$ 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  $\mu$ 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 :  $1 \times 10^6$  cells per test.  
Antibody Concentration Used: 0.5  $\mu$ g/ $10^6$  cells.  
Isotypic Control: FITC Mouse IgG2a

Cell Source Percentage of cells stained above control:

Thymus: 12.1%  
Spleen: 52.0%  
Lymph Node: 35.2%

Strain Distribution by Flow Cytometry Analysis:

Antibody Concentration: 0.5  $\mu$ g/ $10^6$  cells.  
Strains Tested: See Figure 2.

Product images:

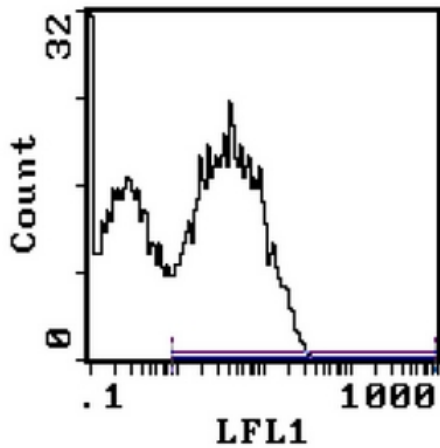


Figure 1. Cell Source: Spleen. Percentage of cells stained above control: 52.0%

Strain	H-2 Loci Alleles								+/-
	<u>K</u>	<u>A<sub>β</sub></u>	<u>A<sub>α</sub></u>	<u>E<sub>β</sub></u>	<u>E<sub>α</sub></u>	<u>C4</u>	<u>C4S</u>	<u>D</u>	
C57BL/6	b	b	b	b	b	b	b	b	+
C3H/He	k	k	k	k	k	k	k	k	-
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.