

Product datasheet for **AM08081BT-S**

MHC Class I H2 Kd/Dd Mouse Monoclonal Antibody [Clone ID: 34-1-2S]

Product data:

Product Type:	Primary Antibodies
Clone Name:	34-1-2S
Applications:	FC
Recommended Dilution:	Flow Cytometry (See Protocols for more details).
	Results:
	<u>Tissue Distribution by Flow Cytometry Analysis:</u>
	Mouse Strain: BALB/c
	Cell Concentration : 1x10 ⁶ cells per test
	Antibody Concentration Used: 0.5 µg/10 ⁶ cells.
	Isotypic Control: Biotin Mouse IgG2a
	<u>Cell Source: Percentage of cells stained above control</u>
	Thymus: 82.5%
	Spleen: 95.7%
	Lymph Node: 100%
Reactivity:	Mouse
Host:	Mouse
Isotype:	IgG2a
Clonality:	Monoclonal
Immunogen:	BDF splenocytes. Donor: C3H spleen. Fusion Partner: Sp2/0-Ag14.
Specificity:	This Monoclonal Antibody reacts with both H-2Kd and H-2Dd products. The antibody also cross reacts with Kbsrpq. This K-D cross reaction indicates the presence of shared specificities between the two separate H-2 regions.
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



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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. This product is photosensitive and should be protected from light. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Background:	The 'classical' MHC Class I molecules are histocompatibility antigens encoded by the H-2 gene complex and consist of heterodimers of highly polymorphic alpha chains noncovalently associated with the invariant beta 2-Microglobulin. (Ref.3,4) These antigens are expressed on most nucleated cells but expression varies on different cell types. MHC Class I molecules present endogenously synthesized peptides to CD8+ T lymphocytes, which are usually cytotoxic T cells. (Ref.5) MHC Class I antigens expressed on thymic epithelial cells regulate the positive and negative selection of CD8+ T cells during T cell ontogeny. (Ref.3,6)

Note: Strain Distribution by Flow Cytometry Analysis:
Antibody Concentration: 0.2 µg/10⁶ cells.
Strains Tested (Figure 2): See Ref.9 for a more detailed strain distribution.

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 2x10⁷ 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 AM08081BT-S per 10⁶ 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).

Product images:

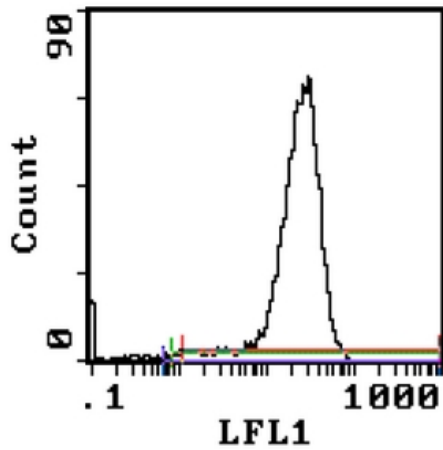


Figure 1. Cell Source: Spleen. Percentage of cells stained above control: 95.7%

Strain	H-2 Loci Alleles	+/-
	<u>K</u> <u>A_β</u> <u>A_α</u> <u>E_β</u> <u>E_α</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.