

## Product datasheet for **CL090F**

### MHC Class II I-Ad Mouse Monoclonal Antibody [Clone ID: 34-5-3S]

#### Product data:

Product Type:	Primary Antibodies
Clone Name:	34-5-3S
Applications:	FC
Recommended Dilution:	Flow cytometry.
Reactivity:	Mouse
Host:	Mouse
Isotype:	IgG2a
Clonality:	Monoclonal
Immunogen:	BDF spleen Donor: C3H/He spleen Fusion Partner: SP2/0-Ag14
Specificity:	This cytotoxic monoclonal antibody specific for cells expressing the Ia antigen coded for by the A subregion of the d, b, p, and q haplotypes. (ie. I-Ad,b,p,q).
Formulation:	PBS, 0.02% NaN <sub>3</sub> and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml. Label: FITC State: Liquid purified Ig
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.
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 0.2 - 0.1  $\mu$ g\* of this Ab 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: BALB/c

Cell Concentration:  $1 \times 10^6$  cells per test

Antibody Concentration Used: 0.1  $\mu$ g/ $10^6$  cells

Isotypic Control: FITC Mouse IgG2a

**Cell Source - Percentage of cells stained above control:**

Spleen: 58.7%

Lymph Node: 23.4%

Thymus: 53.9%

**Strain Distribution by Flow Cytometry Analysis:**

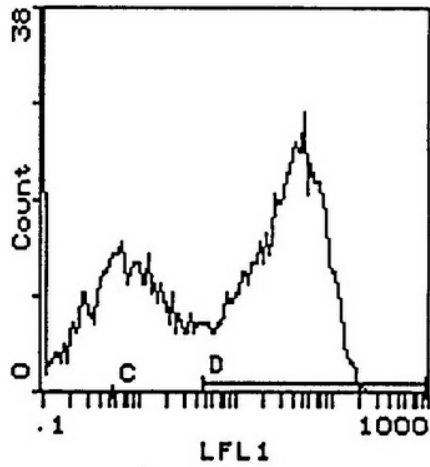
Antibody Concentration: 0.2  $\mu$ g/ $10^6$  cells

Strains Tested: A.TH, A.TL, C3H/He, C57BL/6, DBA/1

Positive: C57BL/6, DBA/1

Negative: A.TH, A.TL, C3H/He

Product images:



Cell Source: Spleen - Percentage of cells stained above control: 58.7%