

Product datasheet for **CL090B**

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 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 ₃ 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 0.2-0.5 μ 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.
 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: BALB/c

Cell Concentration : 1×10^6 cells per tests

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

Isotypic Control: Biotin Mouse IgG2a

Cell Source - Percentage of cells stained above control:

Spleen: 47.0%

Lymph Node: 21.3%

Thymus: 24.6%

Strain Distribution by Flow Cytometry Analysis:

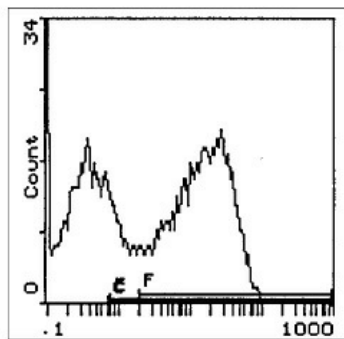
Antibody Concentration Used: 0.2 μ g/ 10^6 cells

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

Positive: C57BL/6, DBA/1

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

Product images:



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Cell Source: Spleen

Percentage of cells stained above control:47.0%