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Product datasheet for SM080B

MHC Class II I-Ab Mouse Monoclonal Antibody [Clone ID: 25-5-16S]

Product data:

Product Type:	Primary Antibodies
Clone Name:	25-5-16S
Applications:	FC
Recommended Dilution:	Flow Cytometry (see protocol below).
Reactivity:	Mouse
Host:	Mouse
Isotype:	IgM
Clonality:	Monoclonal
Specificity:	This antibody specifically reacts with the I-Ab encoded MHC Class II alloantigen expressed on mouse strains BXSB/Mp, C57BL/ 6, C57BL/10, LP/J, and 129. **Tissue Distribution by Flow Cytometry Analysis:** Representative Histogram Mouse Strain: C57BL/6 Cell Concentration : 1x10e6 cells per test Antibody Concentration Used: 0.5 µg/10e6 cells Isotypic Control: Biotin Mouse IgM.
Formulation:	PBS containing 0.09% Sodium Azide 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
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.
Database Link:	P18468
Synonyms:	H2-Eb1, H-2 class II histocompatibility antigen I-A beta chain

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Note:

Protocol: **FLOW CYTOMETRY ANALYSIS:**

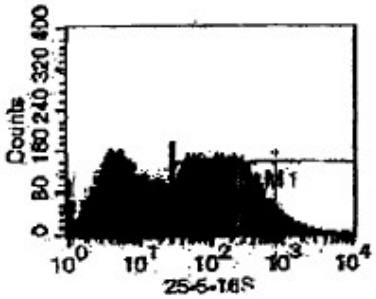
Method:

1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population.
2. Wash 2 times.
3. Resuspend the cells to a concentration of 2x10e7 cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1 x 10e6 cells, representing 1 test).
4. To each tube, add 0.5-1.0 µg of antibody per 10e6 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:



LFL 1
Cell Source: Spleen
Percentage of cells stained above control: 54.7%

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