

## Product datasheet for **SM080P**

### 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:	CT, FC
Recommended Dilution:	Flow cytometry: see protocol.
Reactivity:	Mouse
Host:	Mouse
Isotype:	IgM
Clonality:	Monoclonal
Specificity:	This antibody specifically reacts with the I-Ab encoded MHC class II antigen expressed on mouse strains of the H-2b haplotype.
Formulation:	PBS and 0.09% NaN <sub>3</sub> State: Purified State: Liquid purified Ig fraction
Concentration:	lot specific
Conjugation:	Unconjugated
Storage:	Store the antibody at 2 - 8 °C up to one month or (in aliquots) at -20 °C for longer. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Database Link:	<a href="#">P18468</a>
Background:	Class II antigens are most highly expressed on antigen-presenting cells including B cells, macrophages, dendritic cells and certain epithelial cells.
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  $2 \times 10^7$  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  $\sim 1.0 \mu\text{g}^*$  of antibody.
  5. Vortex the tubes to ensure thorough mixing of antibody and cells.
  6. Incubate the tubes for 30 minutes at  $4^\circ\text{C}$ .
  7. Wash 2 times at  $4^\circ\text{C}$ .
  8. Add 100  $\mu$ l of secondary antibody FITC Goat anti-mouse IgM at 1:500 dilution.
  9. Incubate the tubes at  $4^\circ\text{C}$  for 30-60 minutes. (It is recommended that the tubes are protected from light since most fluorochromes are light sensitive).
  10. Wash 2 times at  $4^\circ\text{C}$  in media B.
  11. Resuspend the cell pellet in 50  $\mu$ l ice cold media B.
  12. 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).
- N.B Appropriate control samples should always be included in labelling studies.
- \* For optimal results in various applications, it is recommended that each investigator determine dilutions appropriate for individual use.