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Monoclonal Antibody to MHC Class I H-2Dk - Purified

Catalog No.:	SM072P
Quantity:	0.2 mg
Concentration:	0.2 mg/ml
Host / Isotype:	Mouse / IgM
Recommended Isotype Controls:	SM13P
Clone:	CTDk
Format:	**State:** Liquid purified Ig fraction **Buffer System:** PBS and 0.09% NaN3
Applications:	Flow Cytometry (see protocol). Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.
Specificity:	This antibody is specific for cells expressing the MHC class I, H-2D antigen coded for by the k haplotype. The reaction pattern of this antibody with a panel of in bred and recombinant haplotypes demonstrates that the antibody detects a private determinant of the H-2Dk antigen. It can be used to quantitate cells bearing the H-2Dk antigen from the appropriate strains of mice (i.e. AKR, C3H/He). Class I antigens are expressed on all nucleated cells, platelets and erythrocytes. **Species:** Mouse. Other species not tested.
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. Shelf life: one year from despatch.
General Readings:	1. Ozato K, Mayer N, Sachs DH. Hybridoma cell lines secreting monoclonal antibodies to mouse H-2 and Ia antigens. J Immunol. 1980 Feb;124(2):533-40. PubMed PMID: 7188699.
Protocols:	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 2x10e cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain 1x10e cells, representing 1 test). 4. To each tube, add ~1.0 µg of antibody. 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 FITC Goat anti-mouse IgM (H+L) at recommended dilution.
9. Incubate the tubes at 4°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°C in media B.
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).