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Monoclonal Antibody to MHC Class I H-2 Dd - PE

Alternate names: D-D alpha chain, H-2 class I histocompatibility antigen, H-2D(D), H2-D1

Catalog No.: CL053R

Quantity: 50 µg

Concentration: 0.1 mg/ml

Uniprot ID: P01900

NCBI: XP_003086970.1

GeneID: 100045864

Host / Isotype: Mouse / IgG2a

Clone: 34-5-8S

Immunogen: Recipient: C3H/HeJ
Immunocyte Donor: B6 x DBA/2 spleen cells
Fusion Partner: SP2/0.Ag14

Format: **State:** Liquid purified Ig
Purification: Protein G Chromatography
Buffer System: PBS, 0.02% NaN3 and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml.
Label: PE

Applications: Flow cytometry.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This monoclonal antibody is specific for cells expressing the H-2D antigen coded for by the d haplotype. The reaction pattern of this antibody with a panel of inbred and recombinant haplotypes demonstrates that the antibody detects a private determinant (H-2.4) of the H-2Dd antigen. This antibody can be used to quantitate cells bearing the H-2Dd (H-2.4) antigen from the appropriate strains of mice.
Species: Mouse.
Other species not tested.

Storage: Store the antibody undiluted at 2-8°C.
DO NOT FREEZE!
This product is photosensitive and should protected from light.
Shelf life: one year from despatch.

General References: Ozato, K., Mayer, N.M., and David H. Sachs. Monoclonal Antibodies to Mouse Major Histocompatibility Complex Antigens. IV. A series of Hybridoma Clones producing Anti-H-2d Antibodies and an Examination of Expression of H-2d Antigens on the Surface of these Cells. Transplantation. 34:113-120 (1982).

Protocols:

FLOW CYTOMETRY ANALYSIS:

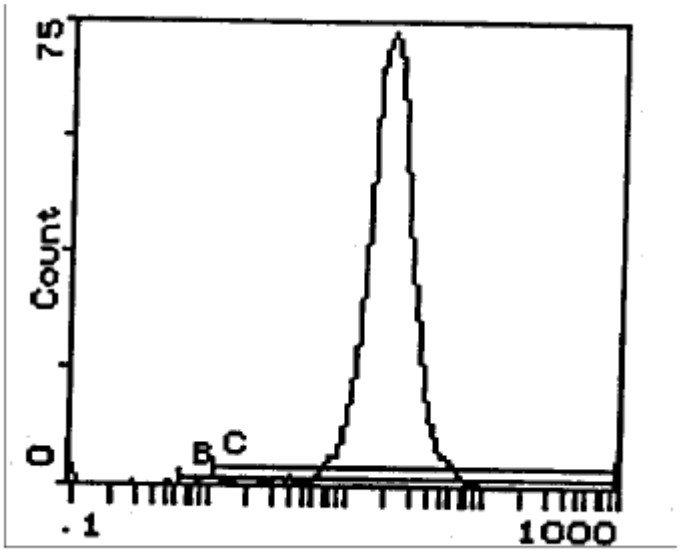
Method:
1. Prepare cell suspension in Media A. For cell preparations, deplete the red blood cell population with Lympholyte®-Mouse cell separation media.
2. Wash 2 times.
3. Resuspend the cells to a concentration of 2x10e7 cells/ml in media A. Add 50µl of this suspension to each tube (each tube will then contain 1 x 10e6 cells, representing 1 test).
4. To each tube add 0.2- 0.5ug of this Ab 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. Resuspend the cell pellet in 50 µl ice cold Media B.
9. Transfer to suitable tubes for flow cytometric analysis containing 15 µl of propidium iodide at 0.5 mg/ml in phosphate buffered saline. (This stains dead cells by intercalating DNA.)

MEDIA:
A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 µl of 2 M sodium azide in 100 mls).
B. Phosphate buffered saline (pH 7.2) + 0.5% bovine serum albumin + sodium azide (100 µl of 2 M sodium azide in 100 mls).

FLOW CYTOMETRIC ANALYSIS:
Mouse Strain: BALB/c
Cell Concentration: 1x10e6 cells per test
Antibody Concentration: 0.5ug /10e6 cells
Isotypic Control: PE Mouse IgG2a

Strain Distribution by Flow Cytometry Analysis:
Cell Concentration: 1x10e6 cells per test
Antibody Concentration Used: 0.5 µg/10e6 cells
Strains Tested: BALB/c, C57BL/6, CBA/J, C3H/He
Positive: BALB/c
Negative: C57BL/6, CBA/J, C3H/He

Pictures:



LFL2 - Cell source: Spleen - Percentage of cells stained above control: 98.2%