Purified Anti-Mouse H-2K^{d} Monoclonal Antibody

Catalog Number: MBS520328

LOT:

DESCRIPTION:

The Purified Anti-Mouse H-2K^{d} Monoclonal Antibody is specific for cells expressing the H-2K 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-2.31) of the H-2K^{d} antigen.

This antibody can be used to quantitate or eliminate cells bearing the H-2K^{d} (H-2.31) antigen from the appropriate strains of mice.

PRESENTATION:

250 ug purified Ig buffered in PBS containing 0.02% sodium azide (NaN_3).

STORAGE/STABILITY:

Store at 4°C. For long term storage, aliquot and freeze unused portions at -20°C in volumes appropriate for single usage. Avoid repeated freeze/thaw cycles.

SPECIFICATIONS:

Clone: 31-3-4S

Hybridoma Production:

Immunization: Immunogen: BALB/c
Donor: C3H/He spleen
Fusion Partner: myeloma SP2/0. Ag 14

Specificity: H-2K^{d}, determinant H-2.31 (private)

Ig Class: Mouse IgM

Format: Purified Ig buffered in PBS containing 0.02% sodium azide (NaN_3).

Antibody Concentration: 1.0 mg/ml

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 $2x10^7$ cells/ml in media A. Add 50 µl of this suspension to each tube (each tube will then contain $1x10^6$ cells, representing 1 test).
4. To each tube, add ~ µg***** of **MBS520328**.
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 µl of secondary antibody **CLCC** () at 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).

Results:

Tissue Distribution by Flow Cytometry Analysis:
Mouse Strain:
Cell Concentration : $1x10^6$ cells per test
Antibody Concentration Used: µg/10^6 cells
Isotypic Control:

N.B Appropriate control samples should always be included in any labelling studies.

*** For optimal results in various applications, it is recommended that each investigation determine dilutions appropriate for individual use.**

Strain Distribution by Cytotoxicity Analysis:

Procedure:
Antibody Concentration Used:
Strains Tested:
Positive:
Negative:

References:

1. Rozera, C., et al., 1999. American Journal of Pathology. 154: 1211-1222.
2. Serreze, D.V., et al., 1998. The Journal of Immunology. 160: 1472-1478.
3. Rovero, S., et al. 2000. The Journal of Immunology. 165:5133-5142.
4. Ozato, K., et al. 1982. Transplantation. 34(3): 113-120.