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Product datasheet for CL060B

MHC Class I H-2Ld Mouse Monoclonal Antibody [Clone ID: 30-5-7S]

Product data:

Product Type:	Primary Antibodies
Clone Name:	30-5-7S
Applications:	FC
Recommended Dilution:	Flow Cytometry (See Protocol below).
Reactivity:	Mouse
Host:	Mouse
Isotype:	IgG2a
Clonality:	Monoclonal
Specificity:	This antibody antibody detects the public specificity H-2.65 of the H-2Ld antigen. It also recognizes H-2Dq and H-2Lq molecules.
Formulation:	PBS containing 0.02% Sodium Azide as preservative and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml. Label: Biotin State: Liquid purrified Ig fraction.
Concentration:	lot specific
Purification:	Protein G Chromatography.
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:	P01897
Background:	The *classical* MHC Class I molecules are histocompatibility antigens encoded by the H-2 gene complex and consist of heterodimers of highly polymorphic alpha chains noncovalently associated with the invariant Beta2-microglobulin. These antigens are expressed on most nucleated cells but expression varies on different cell types. MHC Class I molecules present endogenously synthesized peptides to CD8+ T lymphocytes, which are usually cytotoxic T cells. MHC Class I antigens expressed on thymic epithelial cells regulate the positive and negative selection of CD8+ T cells during T cell ontogeny.

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Synonyms: H2-L

Note: Protocol: **FLOW CYTOMETRY ANALYSIS:**

Method:

1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population with cell separation medium.
2. Wash 2 times.
3. Resuspend the cells to a concentration of 2x10e7cells/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 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).

Results:

Tissue Distribution by Flow Cytometry Analysis:
Mouse Strain: Balb/c
Cell Concentration : 1x10e6 cells per test
Antibody Concentration Used: 1.0 µg/10e6 cells
Isotypic Control: Biotin Mouse IgG2a

Cell Source Percentage of cells stained above control:(See Figure 1.)
Spleen 91.1%
Thymus 99.4%
Lymph Node 98.1%

Strain Distribution:

Strains Tested:
Strain Haplotype + / -
BALB/c H-2d +
A.TH H-2KsDd +
A.TL H-2KsDd +

B.10A(3R) H-2KbDd +
C3H/He H-2k -
C57BL/6 H-2b -

Product images:

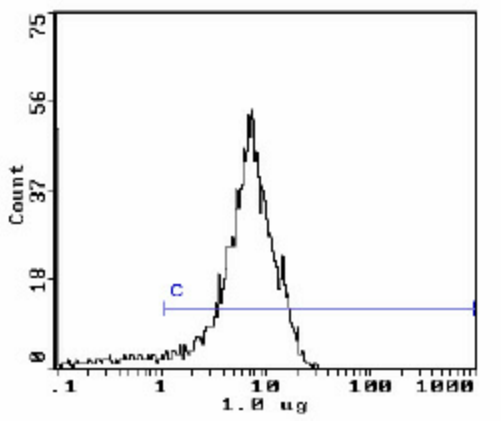

Figure 1. Cell Source: Spleen. Percentage of cells stained above control: 91.1%