

## Monoclonal Antibody to MHC Class I H-2 Dd - FITC

<b>Alternate names:</b>	D-D alpha chain, H-2 class I histocompatibility antigen, H-2D(D), H2-D1
<b>Catalog No.:</b>	CL053F
<b>Quantity:</b>	0.1 mg
<b>Concentration:</b>	0.1 mg/ml
<b>Uniprot ID:</b>	<a href="#">P01900</a>
<b>NCBI:</b>	<a href="#">XP_003086970.1</a>
<b>GeneID:</b>	<a href="#">100045864</a>
<b>Host / Isotype:</b>	Mouse / IgG2a
<b>Clone:</b>	34-5-8S
<b>Immunogen:</b>	Recipient: C3H/HeJ Immunocyte Donor: B6 x DBA/2 spleen cells Fusion Partner: SP2/0.Ag14
<b>Format:</b>	<b>State:</b> Liquid purified Ig <b>Purification:</b> Protein G Chromatography <b>Buffer System:</b> PBS, 0.02% NaN <sub>3</sub> and EIA grade BSA as a stabilizing protein to bring total protein concentration to 4-5 mg/ml. <b>Label:</b> FITC
<b>Applications:</b>	Flow cytometry. Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.
<b>Specificity:</b>	This anti-mouse H-2Dd 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. <b>Species:</b> Mouse. Other species not tested.
<b>Storage:</b>	Store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer. Avoid repeated freezing and thawing. This product is photosensitive and should be protected from light. Shelf life: one year from despatch.
<b>General References:</b>	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).

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Material Safety Datasheets are available at [www.acris-antibodies.com](http://www.acris-antibodies.com) or on request.

Antibody Hotline - Technical Questions - Antibody Location Service  
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**Protocols:****FLOW CYTOMETRY ANALYSIS:****Method:**

1. Prepare cell suspension in Media A. For cell preparations, deplete the red blood cell population with Lympholyte®-M cell separation media.
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 0.5-1.0 mg of this Ab per  $10^6$  cells.
5. Vortex the tubes to ensure thorough mixing of antibody and cells.
6. Incubate the tubes for 30 minutes at 4°C. (It is recommended that tubes are protected from light since most fluorochromes are light sensitive).
7. Wash 2 times at 4°C.
8. Resuspend the cell pellet in 50  $\mu$ l ice cold Media B.
9. Transfer to suitable tubes for flow cytometric analysis containing 15  $\mu$ l 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  $\mu$ l of 2 M sodium azide in 100 mls).
- B. Phosphate buffered saline (pH 7.2) + 0.5% bovine serum albumin + sodium azide (100  $\mu$ l of 2 M sodium azide in 100 mls).

**Results - Tissue Distribution by Flow Cytometry Analysis:**

Donor: BALB/c

Cell Concentration:  $1 \times 10^6$  cells per test

Antibody Concentration: 1  $\mu$ g/ $10^6$  cells

Isotypic Control: FITC Mouse IgG2a, k

**STRAIN DISTRIBUTION:**

Procedure: As above

Antibody Concentration: 1  $\mu$ g/ $10^6$  cells

Strains Tested: see Figure 2

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Pictures:

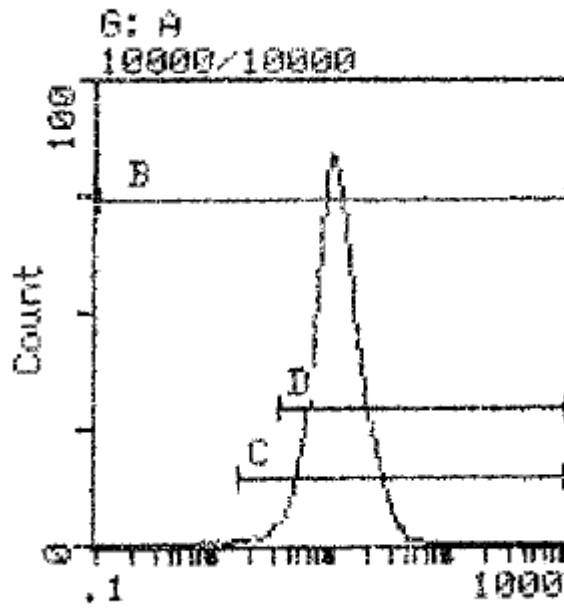


FIGURE1: LFL1 - Cell Source: Spleen - Percentage of Cells Stained Above Control: 97.9%

<u>Strain</u>	<u>Haplotype</u>	<u>+/-</u>
BALB/c	H-2 <sup>d</sup>	+
C3H/He	H-2 <sup>k</sup>	-
CBA/J	H-2 <sup>k</sup>	-
C57BL/6	H-2 <sup>b</sup>	-

FIGURE2

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