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Product datasheet for CL053P

LOC102641613 Mouse Monoclonal Antibody [Clone ID: 34-5-8S]

Product data:

Product Type:	Primary Antibodies
Clone Name:	34-5-8S
Applications:	CT
Recommended Dilution:	Cytotoxicity assay. Functional testing.
Reactivity:	Mouse
Host:	Mouse
Isotype:	IgG2a
Clonality:	Monoclonal
Immunogen:	Recipient: C3H/HeJ Donor: B6XDBA/2 spleen cells Fusion Partner: SP2/0.Ag14
Specificity:	This mAb 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 or eliminate cells bearing H-2Dd (H-2.4) antigen from the appropriate strains of mice.
Formulation:	PBS and 0.02% NaN3 State: Purified State: Liquid purified Ig
Concentration:	lot specific
Purification:	Protein G Chromatography
Conjugation:	Unconjugated
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.
Gene Name:	H-2 class I histocompatibility antigen, D-D alpha chain

View online »

Database Link: Entrez Gene 102641613 Mouse
P01900

Synonyms: H2-D1, H-2D(D)

Note: This reagent is not sold as sterile, but can be sterilized by filtration if necessary. To minimize loss of volume during filtration, first dilute to the final working concentration in the appropriate medium and then filter through a 0.22 µ Millipore filter (or equivalent).

Protocol: **RECOMMENDED METHOD FOR DEPLETING A CELL POPULATION OF H-2Dd BEARING LYMPHOCYTES:**

1. Prepare a cell suspension from the appropriate tissue in Cytotoxicity Medium1 or equivalent. Remove red cells and dead cells (where necessary) by purification of viable lymphocytes on Lympholyte®-M density cell separation medium. After washing, adjust the cell concentration to 1x10e7 cells per ml in Cytotoxicity Medium. 2 Add the antibody to a final concentration of 1:80 and mix. Alternatively, pellet the cells and resuspend in antibody diluted 1:80 in Cytotoxicity Medium.
3. Incubate for 60 minutes at 4°C.
4. Centrifuge to pellet the cells and discard the supernatant.
5. Resuspend to the original volume in Low-Tox®-M Rabbit Complement, diluted to the appropriate concentration in Cytotoxicity Medium. (Recommended concentration included with each batch of Low-Tox®-M Rabbit Complement.)
6. Incubate for 60 minutes at 37°C.
7. Monitor for percent cytotoxicity at this stage, before further processing. For this purpose remove a small sample from each tube, dilute 1:10 with medium, and add 1/10 volume of 1% Trypan Blue. After 3-5 minutes, score live versus dead cells in a haemacytometer.
8. For functional studies, remove the dead cells from the treated groups before further processing, particularly if the treated cells are to be cultured. This can be done by layering the cell suspension separation medium and centrifuging at room temperature as per the instructions provided. Live cells will form a layer at the interface, while the dead cells pellet. The interface can then be collected and washed in Cytotoxicity Medium before being resuspended in the appropriate medium for further processing. Alternately, the cells can be washed and resuspended in the appropriate medium for further processing immediately after Step 6., provided that the dead cells will not interfere with subsequent assays.

RECOMMENDED METHOD FOR DETERMINING PERCENT OF H-2Dd BEARING CELLS IN A POPULATION:

1. Prepare a cell suspension from the appropriate tissue in Cytotoxicity Medium1 or equivalent. Remove red cells and dead cells (where necessary) by purification of viable lymphocytes on Lympholyte®-M density cell separation medium. After washing, adjust the cell concentration to 1x10e6 cells per ml in Cytotoxicity Medium.
2. Add the antibody to a final concentration of 1:80 and mix.
3. Incubate for 60 minutes at 4°C.
4. Centrifuge to pellet the cells and discard the supernatant.

5. Resuspend to the original volume in Low-Tox®-M Rabbit Complement3 diluted to the appropriate concentration in Cytotoxicity Medium. (Recommended concentration included with each batch of Low-Tox®-M Rabbit Complement.
6. Incubate for 60 minutes at 37°C.
7. Place on ice.
8. Add Trypan Blue, 10% by volume of 1% Trypan Blue (w/v) added 3-5 minutes before scoring works well. Score live versus dead cells in a haemacytometer. Cytotoxic Index (C.I.) can be calculated as shown in figure 1.

NOTES:

1. Cytotoxicity Medium is RPMI-1640 with 25mM Hepes buffer and 0.3% bovine serum albumin (BSA). BSA is substituted for the conventionally used fetal calf serum (FCS) because we have found that many batches of FCS contain complement dependent cytotoxins to mouse lymphocytes, thus increasing the background killing in the presence of complement. We recommend that cells not be exposed to FCS prior to or during exposure to antibody and complement. Some batches of BSA also contain complement dependent cytotoxins, resulting in the same problem. We screen for batches of BSA giving low background in the presence of complement and use the selected BSA for preparing Cytotoxicity Medium.
2. Lympholyte®-M cell separation medium is density separation medium designed specifically for the isolation of viable mouse lymphocytes. This separation medium provides a high and non-selective recovery of viable mouse lymphocytes, removing red cells and dead cells. The density of this medium is 1.087-1.088. Isolation of mouse lymphocytes on cell separation medium of density 1.077 will result in high and selective loss of lymphocytes and should be avoided.
3. Rabbit serum provides the most potent source of complement for use with antibodies to mouse cell surface antigens. However, rabbit serum itself is very toxic to mouse lymphocytes. Low-Tox®-M Rabbit Complement is absorbed to remove toxicity to mouse lymphocytes, while maintaining its high complement activity. When used in conjunction with Cytotoxicity Medium, this reagent provides a highly potent source of complement with minimal background toxicity.

ANTIBODY TITRATION:

Cell Source: Splenocytes
Donors: BALB/c
Cell Concentration: 1x10e6 cells per ml
Complement: Low-Tox®-M Rabbit Complement
Complement Concentration: 1:10
Procedure: Two stage cytotoxicity as described on: Recommended Method for Determining Percent of H-2Dd Bearing Cells in a Population.
SEE FIGURE 2

STRAIN DISTRIBUTION:

Procedure: As above
Antibody Concentration: Final concentration 1:80

Strains Tested: see Figure 3

TISSUE DISTRIBUTION:
Procedure: As above
Antibody Concentration: 1:80
Strain: BALB/c

Cell Source C.I.
Spleen: 71
Thymus: 24
Lymph Node: 57
Bone Marrow: 67

FUNCTIONAL TESTING:
Cell Source: Spleen
Donors: BALB/c, C3H/He
Cell Concentration: 1x10e7 cells/ml
Antibody Concentration: 1:80
Complement: Low-Tox®-M Rabbit Complement
Complement Concentration: 1:10

PROCEDURE:
Cells were treated as described in Recommended Method for Depleting a Cell Population of H-2Dd Bearing Lymphocytes. Treated cells and controls were tested for:
a) the ability to generate plaque-forming cells (PFC) using a modified Jerne haemolytic plaque assay and
b) the ability to generate cytotoxic T effector cells using a cytotoxic lymphocyte reaction (CTL) assay.
Cells were treated both before and after sensitization in the CTL assay. In vitro immunizations were used in all experiments.

RESULTS:
Treatment of BALB/c splenocytes with anti-H-2Dd plus complement resulted in a marked reduction in the number of plaque-forming cells and essentially eliminated cytotoxic T cells, as assessed by Jerne haemolytic plaque assay and CTL assay, respectively. Treatment of C3H/He splenocytes had no effect. These results are consistent with the depletion of H-2Dd bearing cells.

Product images:

$$\text{C.I.} = 100 \times \frac{\%\ \text{cyt. (antibody + complement)} - \%\ \text{cyt. (complement alone)}}{100 - \%\ \text{cyt. (complement alone)}}$$

Figure 1

Strain	Haplotype	+/-
C3H/He	H-2^k	-
CBA/J	H-2^k	-
BALB/c	H-2^d	+
SWR/J	H-2^q	-
SJL/J	H-2^s	-
ATH	H-$2K^sD^d$	+
ATL	H-$2K^sD^d$	+

Figure 3

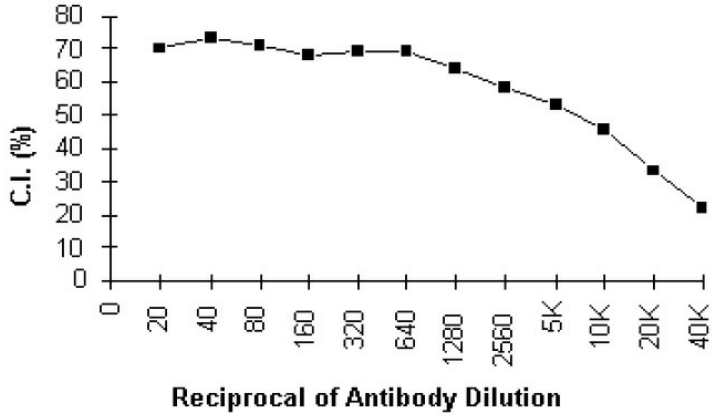

Figure 2