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CL059 Monoclonal Antibody to MHC Class I H-2 Kd / H-2 Dd - Ascites

Alternate names: H2 class I histocompatibility antigen, MHC H2D, MHC H2K

Quantity: 0.5 ml

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 beta 2-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.

Host / Isotype: Mouse / IgM

Clone: 34-7-23S

Immunogen: B6 X DBA/2 spleen cells
Donor: C3H/He
Fusion Partner: Myeloma SP2/0.Ag 14

Format: **State:** Lyophilized Ascites filtered to 0.45 µm (non-sterile).
Reconstitution: Restore with 0.5 ml of cold distilled water.

Applications: Cytotoxicity Analysis.
Functional assay.
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-2K and/or 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 previously unidentified serological specificity similar to H-2.27, H-2.28, H-2.29. This antibody can be used to quantitate or eliminate cells bearing the H-2Kd and/or H-2Dd antigen from the appropriate strains of mice. This antibody will also react with H-2Kb and H-2q.
Species: Mouse.
Other species not tested.

Storage: Prior to reconstitution store at 2-8°C.
Following reconstitution store the antibody at -20°C.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

Protocols: **CYTOTOXICITY ANALYSIS:**

Method:
1. Prepare a cell suspension from the appropriate tissue in Cytotoxicity Mediuma or equivalent. Remove red cells and dead cells (where necessary) by purification of viable lymphocytes on Lympholyte®-Mb density cell separation medium. After

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Material Safety Datasheets are available at www.acris-antibodies.com or on request.

washing, adjust the cell concentration to 1x10e6 cells per ml in Cytotoxicity Medium.
2. Add the antibody to a final concentration of 1: 50 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 Complementc diluted to the recommended concentration in Cytotoxicity Medium..
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 hemacytometer.
Cytotoxic Index (C. I.) can be calculated as shown in figure 1.

Results - Antibody Titration by Cytotoxicity Analysis:
See figure 2
Cell Source: Splenocytes
Donor: BALB/c
Cell Concentration: 1.1x10e6 cells/ml
Complement: Low-Tox®-M Rabbit Complement
Complement Concentration: 1:10
Procedure: Two-stage cytotoxicity

Tissue Distribution by Cytotoxicity Analysis:
Antibody Concentration Used: 1:500
Strain: BALB/c

Cell Source - C.I.
Spleen: 72
Thymus: 20
Lymph Node: 80
Bone Marrow: 79

Strain Distribution by Cytotoxicity Analysis:
Antibody Concentration Used: 1:40
Strains Tested: C57BL/6, C3H/He, CBA/J, BALB/c, A.TL, ATH
Positive: C57BL/6, BALB/c, A.TL, ATH
Negative: C3H/He, CBA/J

CYTOTOXICITY DEPLETION ASSAY:

Method:
1. Prepare a cell suspension from the appropriate tissue in Cytotoxicity Mediuma 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 1:25 and mix. Alternatively, pellet the cells and resuspend in antibody diluted 1:25 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 Complementc, 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 hemacytometer.
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 over a 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. Alternatively, 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.

FUNCTIONAL TESTING:

Method:
Cells were treated as described in Cytotoxicity Depletion Assay. Treated cells and controls were tested for:
a) the ability to generate plaque-forming cells (PFC) using a modified Jerne haemolytic plaque assay.
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:
Cell Source: Splenocytes
Donors: C57BL/6 and C3H/He.
Cell Concentration: 1x10e7 cells/ml
Antibody Concentration Used: 1:25
Complement: Low-Tox®-M Rabbit Complement
Complement Concentration Used: 1:10
Treatment of C57BL/6 splenocytes with this Ab plus complement resulted in a significant reduction in the number of plaque-forming cells. As assessed by a CTL assay, cytotoxic T cell function was essentially eliminated in both presensitized and post sensitized treated samples. No effect was observed when C3H/He cells were used. These results are consistent with the removal of T helper and T cytotoxic activity.

NOTES:

a. Cytotoxicity Medium is RPMI-1640 with 25 mM 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. 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.
b. Lympholyte®-M cell separation medium is a 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.
c. 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.

Pictures:

Figure 2.

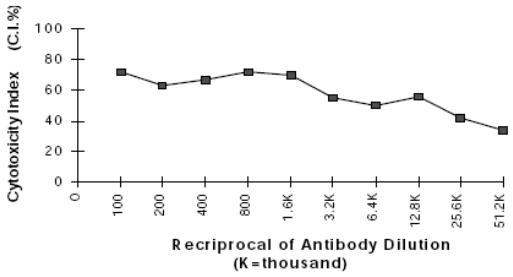

Figure 1.

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