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TECHNICALLY Speaking

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

Anti-Mouse H-2D^{b} Monoclonal Antibody
Ascites

CL9020A
LOT: 2011

DESCRIPTION:

Cedarlane Anti H-2D^{b} monoclonal antibody reacts with H-2D^{b} products. This antibody has been shown to cross react, most notably, with H-2D^{d}.

Application: Cytotoxicity Assays

PRESENTATION: 0.5 ml, lyophilized

STORAGE/STABILITY:

Store at -20°C or below before reconstitution. Reconstitute with 0.5 ml of distilled water. For long term storage, aliquot and freeze unused portions at -20°C in volumes appropriate for single usage. Avoid repeated freeze/thaw cycles.

STERILITY:

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 µm filter.

RECOMMENDED METHOD FOR DEPLETING A CELL POPULATION OF H-2D^{b} BEARING LYMPHOCYTES:

1. Prepare a cell suspension from the appropriate tissue in Cedarlane Cytotoxicity Medium[1] or equivalent. Remove red cells and dead cells (where necessary) by purification of viable lymphocytes on Cedarlane Lympholyte®-M density cell separation medium. After washing, adjust the cell concentration to 1x10^{7} cells per ml in Cytotoxicity Medium.

2 Add the antibody to a final concentration of 1:250 and mix. Alternatively, pellet the cells and resuspend in antibody diluted 1:250 in Cytotoxicity Medium.

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For more information or to place an order please contact...

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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 Cedarlane 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 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 treated cell suspensions over an equal volume of Lympholyte® -M 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-2D^{b} BEARING CELLS IN A POPULATION:

1. Prepare a cell suspension from the appropriate tissue in Cedarlane Cytotoxicity Medium[1] or equivalent. Remove red cells and dead cells (where necessary) by purification of viable lymphocytes on Cedarlane Lympholyte®-M density cell separation medium. After washing, adjust the cell concentration to $1x10^6$ cells per ml in Cytotoxicity Medium.

2. Add the antibody to a final concentration of 1:320 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 Cedarlane Low-Tox®-M Rabbit Complement[3] 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 hemacytometer. Cytotoxic Index (C.I.) can be calculated as follows:

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

NOTES:

1. Cedarlane 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 Cedarlane Cytotoxicity Medium.

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2. Cedarlane 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. Cedarlane Low-Tox®-M Rabbit Complement is absorbed to remove toxicity to mouse lymphocytes, while maintaining its high complement activity. When used in conjunction with Cedarlane Cytotoxicity Medium, this reagent provides a highly potent source of complement with minimal background toxicity.

SPECIFICATIONS:

Clone: 28-11-5S

Hybridoma Production:

Immunization: Immunogen: C3H.SW splenocytes
Donor: C3H splenocytes
Fusion Partner: Sp2/0-Ag14

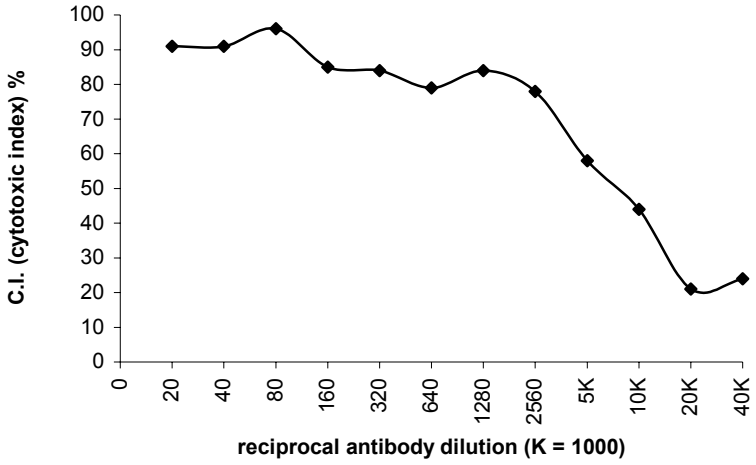
Specificity: Anti H-2D^b

Ig Class: Mouse IgM

Presentation: lyophilized ascites fluid, 0.45μm filtered

ANTIBODY TITRATION:

Cell Source: Spleen
Donors: C57BL/6
Cell Concentration: 1.1x10^6 cells per ml
Complement: Cedarlane Low-Tox®-M Rabbit Complement
Complement Concentration: 1:15
Procedure: Two stage cytotoxicity as described on page 3, "Recommended Method for Determining Percent of H-2D^b bearing cells in a Population".



STRAIN DISTRIBUTION:

Procedure: As above
Antibody Concentration: Final concentration 1:80
Strains Tested: C3H/He, C57BL/6, A.TH, CBA, BALB/c, AKR

Cells Killed by Treatment: C57BL/6, A.TH, BALB/c
Cells not Killed by Treatment: C3H/He, CBA, AKR

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TISSUE DISTRIBUTION:

Procedure: as above
Antibody Concentration: 1:80
Strain: C57BL/6

Cell Source	C.I.
Spleen	96
Thymus	82
Lymph Node	72
Bone Marrow	46

FUNCTIONAL TESTING:

Cell Source: Spleen
Donors: C3H/He, C57BL/6
Cell Concentration: $1x10^7$ cells/ml
Antibody Concentration: 1:500
Complement: Cedarlane 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-2D^b Bearing Lymphocytes", on page 2. 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 C57BL/6 splenocytes with anti H-2D^b plus complement essentially eliminates plaque-forming cells and 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-2D^b bearing cells.

REFERENCES

1) J. Immunol. 126: 317-321, 1981
2) J. Immunol. 166:1601-1610, 2001

FOR RESEARCH USE ONLY

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