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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-2K^{d}
Monoclonal Antibody - Ascites

CL9008A
LOT: 9812

DESCRIPTION:

Cedarlane's Anti-Mouse H-2K^{d} Monoclonal Antibody is specific for cells expressing the H-2K 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-2.31) of the H-2K^{d} antigen. This antibody can be used to quantitate or eliminate cells bearing the H-2K^{d} (H-2.31) antigen from the appropriate strains of mice. This clone has also been reported to work in flow cytometry[1,2,3].

PRESENTATION:

0.5 ml, lyophilized ascites.

STORAGE/STABILITY:

Lyophilized form stable at 4°C or -20°C. Reconstitute with 0.5 ml of cold distilled water. After reconstitution, aliquot and freeze unused portions in volumes appropriate for single usage. Avoid repeated freeze/thaw cycles.

SPECIFICATIONS:

Clone: 31-3-4S

Hybridoma Production:

Immunization: Immunogen: BALB/c
Donor: C3H/He spleen

Fusion Partner: myeloma SP2/0. Ag 14

Specificity: H-2K^{d}, determinant H-2.31 (private)

Ig Class: Mouse IgM

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

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website: www.cedarlanelabs.com • e-mail: info@cedarlanelabs.com

Format: Ascitic fluid, filtered to 0.8 µ (non-sterile) and lyophilized.

Antibody Concentration: 0.94 mg/ml (as determined by RID)

CYTOTOXICITY ANALYSIS:

Method:

1. Prepare a cell suspension from the appropriate tissue in Cedarlane Cytotoxicity Medium[a] or equivalent. Remove red cells and dead cells (where necessary) by purification of viable lymphocytes on Cedarlane Lympholyte®-M[b] 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 1:400 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[c] 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 follows:

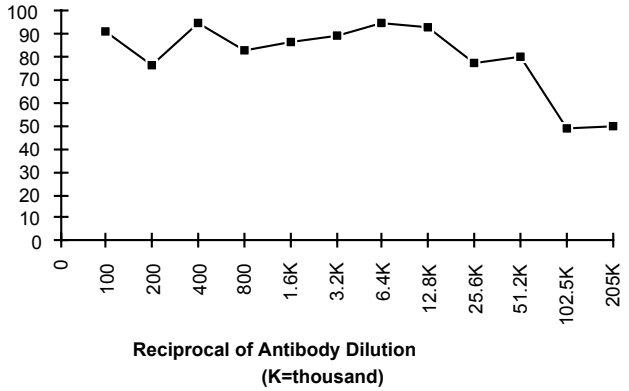
$$\text{C.I.} = 100 \times \frac{\%\text{ cyt (antibody + complement)} - \%\text{ cyt (complement alone)}}{100\% - \%\text{ cyt (complement alone)}}$$

Results:

Antibody Titration by Cytotoxicity Analysis:

Cell Source: Splenocytes
Donor: BALB/c
Cell Concentration: $1.1x10^6$ cells/ml
Complement: Cedarlane Low-Tox®-M Rabbit Complement
Complement Concentration: 1:10
Procedure: Two-stage cytotoxicity as described on page 2

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



Tissue Distribution by Cytotoxicity Analysis:

Procedure: see page 2
Antibody Concentration Used: 1:5000
Strain: BALB/c

Cell Source	C.I.
Spleen	95
Thymus	82
Lymph Node	83
Bone Marrow	90

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Strain Distribution by Cytotoxicity Analysis:

Procedure: see page 2
Antibody Concentration Used: 1:100
Strains Tested: BALB/c, C57BL/6, C3H/He, CBA/J, AKR/J
Positive: BALB/c
Negative: C57BL/6, C3H/He, CBA/J, AKR/J

CYTOTOXICITY DEPLETION ASSAY:

Method:

1. Prepare a cell suspension from the appropriate tissue in Cedarlane Cytotoxicity Medium[a] 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 1:20 and mix. Alternatively, pellet the cells and resuspend in antibody diluted 1:20 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 Cedarlane Low-Tox-M® Rabbit Complement[c], 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" on page 4. 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: BALB/c and C3H/He
Cell Concentration: $1x10^7$ cells/ml
Antibody Concentration Used: 1:20
Complement: Cedarlane Low-Tox®-M Rabbit Complement
Complement Concentration Used: 1:10

Treatment of BALB/c and C3H/He splenocytes with CL9008A 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 postsensitized treated samples. These results are consistent with the removal of T helper and T cytotoxic cell activity.

NOTES:

a. Cedarlane 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

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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 Cedarlane Cytotoxicity Medium.

b. Cedarlane 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. 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.

References:

1. Rozera et al., American Journal of Pathology. 1999; 154: 1211-1222.
2. Serreze et al., The Journal of Immunology. 1998; 160: 1472-1478.
3. Rovero et al., The Journal of Immunology. 2000; 165:5133-5142.

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NWB 11/06/03