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TECHNICALLY Speaking

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

Anti-Ly 1.1 Monoclonal Antibody - Ascites
CD5 equivalent

CL8911A
LOT: 6103

DESCRIPTION:

Cedarlane's Anti-Ly 1.1 monoclonal antibody reacts with T lymphocytes from mouse strains expressing the Ly 1.1 phenotype, but does not react with lymphocytes from mouse strains expressing the Ly 1.2 phenotype.

PRESENTATION:

0.5ml, lyophilized

STORAGE/STABILITY:

Store at –20°C or below before reconstitution. Reconstitute with 1.0ml of distilled water. Aliquot and freeze the unused portion in volumes appropriate for single usage (as repeated freezing and thawing may cause loss of antibody activity).

STERILITY:

This reagent is not sold as sterile, but can be sterilized by filtration if necessary. To minimize loss of volume during filtration, dilute to the final working concentration in the appropriate medium before filtration.

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

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Recommended Method for Depleting a Cell Population of Ly 1.1 Positive Lymphocytes:

1. Prepare a cell suspension from the appropriate tissue in Cedarlane Cytotoxicity Medium[a] (CL95100) or equivalent. Remove red cells and dead cells (where neces sary) by purification of viable lymphocytes on Cedarlane Lympholyte®-M cell sepa ration medium (CL5030). 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.
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] (CL3051) , diluted to the appropriate concentration in Cytotoxicity Medium. (Rec ommended concentration included with each batch of Low-Tox-M® Rabbit Comple ment.)
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.

Recommended Method for Determining Percent of Ly 1.1 Positive Cells in a Popula- tion:

1. Prepare a cell suspension from the appropriate tissue in Cedarlane Cytotoxicity Medium[a] (CL95100) or equivalent. Remove red cells (where necessary) by treating cell pellet with 5 times the volume of 0.17M NH_4Cl-Tris Buffer for 3 minutes at room temperature or by purification on cell separation medium. After washing, adjust cells to 1×10^6 cells/ml in Cytotoxicity Medium[a].
2. Add the anti-RT2 monoclonal antibody to a final concentration of 1:500 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 Cytotoxicity Medium[a] with the appropriate concen- tration of Cedarlane Baby Rabbit Complement (CL3441)[b]
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 x $\frac{\%\text{ cyt (antibody + complement)} - \%\text{ cyt (complement alone)}}{100\% - \%\text{ cyt (complement alone)}}$

SPECIFICATIONS:

Clone: 7-20.6/3

Hybridoma Production:

Immunization: Recipient: 129/ReJ
Donor: B6-Ly-1[a]
Fusion Partner: Spleen from immunized recipient fused with *myeloma P3-NSI-Ag4-1

Specificity: Anti-Ly 1.1

Ig Class: IgG_{2a} cytotoxic

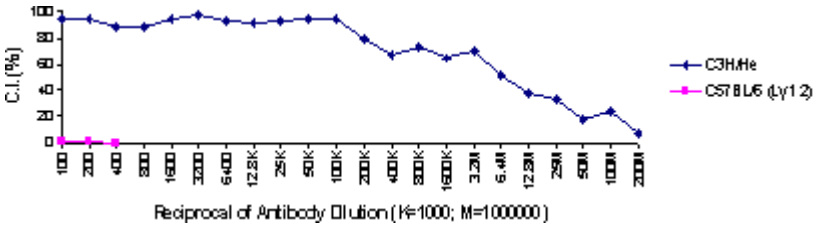
Format: Ascites fluid

Results:

Antibody Titration by Cytotoxicity Analysis:
Cell Source: Thymus
Donors: C3H/He
C57BL/6J (Ly 1.2)
Cell Concentration: $1x10^6$ cells/ml
Complement: Cedarlane Low-Tox-M® Rabbit Complement[b] (CL3051)
Complement Concentration: 1:18
Procedure: Two stage cytotoxicity as described under Recommended Methods for Determining Percent of Ly 1.1 Positive Cells in in a Population.

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

Tissue Distribution:
Procedure: see page 2



Antibody Concentration: 1:500
Strain: C3H/He

Cell Source	C.I.
Thymus	88
Spleen	27
Lymph Node	57

Strain Distribution:
Procedure: See page 3
Antibody Concentration: 1:500
Strains tested:

Strain	Phenotype	+/-
C57BL/6J	Ly 1.2	-
CBA/J	Ly 1.1	+
BALB/c	Ly 1.2	-
C3H/He	Ly 1.1	+
B6Ly1.1	Ly 1.2	+
A.TH	Ly 1.2	-
A.TL	Ly 1.2	-
B.10A(4R)	Ly 1.2	-

FUNCTIONAL ANALYSIS:

Method:

Cells were treated as described in "Recommended Method for Depleting a Cell Population of Ly 1.1 Positive 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
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: $1x10^7$ cells/ml
Antibody Concentration used: 1:10
Complement: Cedarlane Low-Tox®-M Rabbit Complement
Complement Concentration: 1:10

Treatment of C3H/He splenocytes with anti- Ly 1.1 plus complement was found to reduce the number of plaque-forming cells and inhibit cytotoxic T cell generation indicative of the removal of T helper cell activity. Cytotoxic T effector cell function was not affected (cells treated after sensitization). No effect was observed when C57BL/6 cells were used. These results are consistent with the depletion of T helper cells of the Ly 1.1 phenotype.

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 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.

b. 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.

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.

FOR RESEARCH USE ONLY

JB 2002/05/31