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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

TECHNICALLY Speaking

Place your order with CEDARLANE® or your local distributor.

Please contact CEDARLANE® for lot specific information.

Anti-Mouse I-A^{d}
Monoclonal Antibody - Ascites

CL8713A
LOT: 1311

DESCRIPTION:

Cedarlane's CL8713A is a cytotoxic monoclonal antibody specific for cells expressing the Ia antigen coded for by the A subregion of the d, b, p, and q haplotypes. (ie. I-A^{d,b,p,q})

PRESENTATION:

0.5 ml, lyophilized.

STORAGE/STABILITY:

Lyophilized form stable at –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.

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

CEDARLANE® LABORATORIES LIMITED
5516 - 8th Line, R.R.#2, Hornby, Ontario, CANADA L0P 1E0

toll free: 1-800-268-5058
in North America
phone: (905) 878-8891 • fax: (905) 878-7800

or visit our website for a list of our international distributors including contact information
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SPECIFICATIONS:

Clone: 34-5-3s

Hybridoma Production:

Immunization:	Immunogen: BDF splenocytes
	Donor: C3H splenocytes
Fusion Partner:	SP2/0-Ag14

Specificity: Mouse I-A^d

Ig Class: Mouse IgG_{2a}

Presentation: Ascitic fluid filtered to 0.45 µm and lyophilized.

CYTOTOXICITY ANALYSIS:

Method:

1. Prepare a cell suspension from the appropriate tissue in Cedarlane Cytotoxicity Medium[a] (CL95100) or equivalent. Remove red cells and dead cells (where necessary) by purification of viable lymphocytes on Cedarlane Lympholyte®-M[b] (CL5030) 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 1:80 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 Cedarlane Low-Tox®-M Rabbit Complement[c] (CL3051) 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:

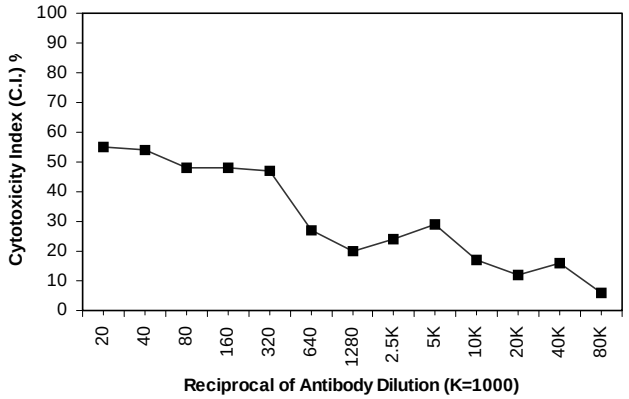
$$\text{C.I.} = 100 \times \frac{\%\ \text{cyt (antibody + complement)} - \%\ \text{cyt (complement alone)}}{100\% - \%\ \text{cyt (complement alone)}}$$

RESULTS:

ANTIBODY TITRATION BY CYTOTOXICITY ANALYSIS:

Cell Source: Splenocytes
Donor: DBA
Cell Concentration: 1.1 x 10^6 cells/ml
Complement: Cedarlane Low-Tox®-M Rabbit Complement (CL3051)
Complement Concentration: 1:10
Procedure: see page 2

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



TISSUE DISTRIBUTION BY CYTOTOXICITY ANALYSIS:

Procedure: see page 2
Antibody Concentration used: 1:40
Strain: DBA

Cell Source:

Thymus	0
Spleen	54
Lymph Node	16
Bone Marrow	12

STRAIN DISTRIBUTION BY CYTOTOXICITY ANALYSIS:

Procedure: As above
Antibody Concentration Used: 1:40
Strains Tested: C57BL/6; C3H/He; DBA; A.TH; A.TL

Cells Killed by Treatment: C57BL/6; DBA
Cells Not Killed by Treatment: C3H/He, A.TH; A.TL

CYTOTOXIC DEPLETION ASSAY:

1. Prepare a cell suspension from the appropriate tissue (e.g. spleen, lymph node, etc.) in Cedarlane Cytotoxicity Medium[a] or equivalent. Remove erythrocytes and dead cells (where necessary) by purification on Cedarlane Lympholyte®-M density cell separation medium[b]. After washing, adjust the cell concentration to $1.1x10^7$ cells per ml in cytotoxicity medium.
2. Add the antibody to a final concentration of 1:40.
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 containing the appropriate concentration of Cedarlane Low-Tox®-M Rabbit Complement[3,4].
6. Incubate for 60 minutes at 37°C.
7. Place on ice and monitor for percent cytotoxicity before further processing. For this purpose, remove a small sample from each tube, dilute 1:10, 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 dead cells from treated groups before further processing, particularly if the treated cells are to be cultured. Layering the treated cell suspension over an equal volume of Cedarlane Lympholyte-M cell separation medium and centrifuging, as per the instructions provided, can do this. Live cells will form a layer at the interface, while the dead cells pellet. The interface can then be collected in cytotoxicity medium before being resuspended in the appropriate medium for further processing.

FUNCTIONAL ANALYSIS:

Method:

Cells were treated as described in "Cytotoxic 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: C3H/He and BALB/c
Cell Concentration: $1x10^7$ cells/ml
Antibody Concentration: 1:100
Complement: Cedarlane Low-Tox®-M Rabbit Complement
Complement Concentration: 1:10

Treatment of BALB/c splenocytes with CL8713A plus complement resulted in a significant reduction in the number of plaque forming cells. Partial inhibition of cytotoxic T effector cell function as assessed by CTL assay was also noted. Treatment of C3H/He cells had no effect on either plaque forming cell number or cytotoxic T cell function. These results are consistent with the removal of $I\text{-}A^d$ bearing cells and their related activities.

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.

REFERENCES:

1. Ozato, K., et al. 1982. Monoclonal Antibodies to Mouse Major Histocompatibility Complex Antigens. Transplantation. **34**: 113-120.
2. Ahn, H.J. et al. 1997. A Mechanism Underlying Synergy Between IL-12 and IFN-γ-Inducing Factor in Enhanced Production of IFN-γ. Journal of Immunology. **159**: 2125-2131.

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