SZABO SCANDIC

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- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand


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TECHNICALLY Speaking

**Place your order with CEDARLANE® or your local distributor.**

*Please contact CEDARLANE® for lot specific information.*

# Anti-Mouse I-A$^{p}$ Ig Fraction Monoclonal Antibody-Ascites

**CL8708A**
**LOT :** 1224

DESCRIPTION: Cedarlane's anti-I-A$^{p}$ monoclonal antibody is a cytotoxic antibody which defines a new public I-A antigen. This antibody reacts with I-A antigen from the following I-A haplotypes: I-A$^{p,k,q,r,s,j,b}$. Using recombinant strains, reactivity against the b haplotype has been localized to the A$^{b}$ subregion. This antibody can be used to quantitate or eliminate I-A bearing cells for precipitating I-A antigen.

PRESENTATION: 1.0 ml, lyophilized

STORAGE AND RECONSTITUTION: Store at -20°C or below before reconstitution. Reconstitute with 1.0 ml 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.

RECOMMENDED METHOD FOR DEPLETING A CELL POPULATION OF I-A ANTIGEN BEARING CELLS:

RECOMMENDED METHOD FOR DETERMINING PERCENT OF I-A ANTIGEN 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 1 x $10^{6}$ 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.

*Continued overleaf….*

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

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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 Cytotoxicty 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 vs dead cells in a hemacytometer. Cytotoxic index (C. I.) can be calculated as follows:

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

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 ®.

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: 7-16.17

HYBRIDOMA PRODUCTION:
Immunization: Recipient: BALB/c
Donor: B10.P
Fusion Partner: Spleen from immunized recipient fused with myeloma SP2/0

SPECIFICITY: Mouse-I-$A^{p,k,q,r,s,j,b}$

Ig CLASS: Mouse $IgG_{2a}$

PRESENTATION: Immunoglobulin fraction of ascites in PBS.

LOT SPECIFICATIONS: LOT NUMBER: 1224

ANTIBODY TITRATION:

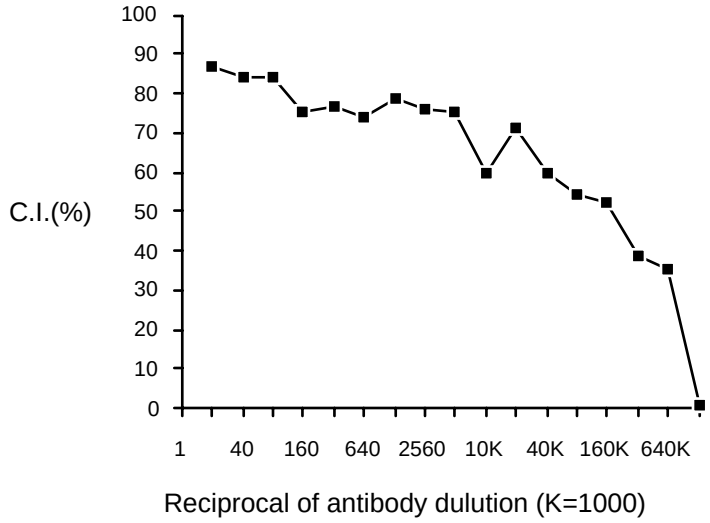
CELL SOURCE: BDP (I-$A^{p}$) "enriched splenic B cells"*
CELL CONCENTRATION: $1 \times 10^{6}$ cells per ml

*Continued overleaf…..*

COMPLEMENT:Cedarlane Low-Tox-M® Rabbit Complement
COMPLEMENT CONCENTRATION: 1:12
PROCEDURE: Two stage cytotoxicity as described on page 3 "Recommended Method for Determining Percent of I-A Antigen Bearing Cells in a Population".

C.I. = Cytotoxic Index =

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




REFERENCES:

1. Harmon, R.C., Stein, N., Frelinger, J.A., 1983. Immunogenetics 18:541-545.

STRAIN DISTRIBUTION:
Procedure: as above
Antibody Concentration: 1:40
Strains tested:

| Strain | Haplotype | +/- |
|---|---|---|
| C57BL/6 | I-$A^b$ | + |
| BALB/c | I-$A^d$ | - |
| C3H/He | I-$A^k$ | + |
| A.TH | I-$A^s$ | + |
| BDP | I-$A^p$ | + |

TISSUE DISTRIBUTION:
Procedure: As above
Antibody Concentration: 1:80
Strain: BDP (I-$A^p$)

| Cell Source | C.I. |
|---|---|
| Spleen | 36 |
| Thymus | 3 |
| Lymph Node | 21 |
| "Enriched Splenic B-cells"* | 86 |

*Enriched Splenic B-cells prepared by treatment of Lympholyte®-M isolated spleen lymphocytes with Cedarlane Monoclonal Anti-Thy-1.2 antibody plus complement, followed by removal of dead cells on Lympholyte®-M.

*Continued overleaf…*

FUNCTIONAL TESTING:
Cell Source: Splenocytes
Donors: BALB/c and C57BL/6
Cell Concentration: 1 x $10^7$ cells/ml
Antibody Concentration: 1:20
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 I-A Antigen 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-I-$A^p$ plus complement resulted in a marked reduction in the number of plaque-forming cells. Partial inhibition of cytotoxic T cell function as assessed by CTL assay was also noted. No effect in either assay was observed when BALB/c cells were used. These results are consistent with the removal of I-$A^p$ bearing cells and their related activities.