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Product datasheet for CL073

MHC Class II I-Ed Mouse Monoclonal Antibody [Clone ID: 34-1-4S]

Product data:

Product Type:	Primary Antibodies
Clone Name:	34-1-4S
Applications:	CT
Recommended Dilution:	Cytotoxicity analysis.
Reactivity:	Mouse
Host:	Mouse
Isotype:	IgM
Clonality:	Monoclonal
Immunogen:	Recipient: C3H Donor: B6xDBA/2
Specificity:	This monoclonal antibody detects the private specificity of the I-Ed antigen (determinant Ia.m23).
Formulation:	State: Ascites State: Lyophilised Ascites.
Reconstitution Method:	Restore with 1.0 ml of distilled water.
Conjugation:	Unconjugated
Storage:	Prior to and following reconstitution store the antibody at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Note:	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 and filter through a 0.45 µ millipore filter (or equivalent). Protocol: **RECOMMENDED METHOD FOR DEPLETING A CELL POPULATION OF I-Ed BEARING LYMPHOCYTES:** **Method:** 1. Prepare a cell suspension from the appropriate tissue in Cytotoxicity Medium® or equivalent. Remove red cells and dead cells (where necessary) by purification of viable

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lymphocytes on Lympholyte-M® denstiy cell concentration to 1 x 10e7 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. If using thymocytes or lymph node cells, resuspend to the original volume in Low-Tox-M® Rabbit Complement diluted 1/10 in cytotoxicity medium. If using splenocytes or bone marrow cells, resuspend to the original volume in Baby Rabbit Complement diluted 1/10 in cytotoxicity medium.
6. Incubate for 60 minutes at 37°C.
7. If desired, 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 vs. dead cells in a hemacytometer.
8. For functional studies, remove the dead cells form 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 an equal volume of Lympholyte-M® cell 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:
Cell Source: Splenocytes
Donors: BALB/c and C3H/He
Cell Concentration: 1 x 10e7 cells/ml
Antibody Concentration: 1:20
Complement: Baby Rabbit Complement
Complement Concentration: 1:10

Procedure:
Cells were treated as described in Recommended Method for Depleting a Cell Population of I-Ed Bearing Lymphocytes. 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 lumphocyte reaction (CTL) assay. Cells were treated both before and after sensitiztion in the CTL assay. In vitro immunizations were used in all experiments.

Results:
Treatment of Balb/c splenocytes with anti-I-Ed plus complement was found to reduce the number of plaque-forming cells. Cytotoxic T cell generation or cytotoxic T effector cell

function was not affected. No effect was observced when C3H/He cells were used. There results are consistent with the depletion of lymphocytes of the I-Ed phenotype.

RECOMMENDED METHOD FOR DETERMINING PERCENT OF I-Ed BEARING CELLS IN A POPULATION:

1. Prepare a cell suspension from the appropriate tissue in cytotoxicity medium® or equivalent. Remove red cells and dead cells (where necessary) by purification of viable lymphocytes on Lympholyte-M® density cell separation medium. After washing, adjust the cells concentration to 1 x 10e6 cells per ml in cytotoxicity medium.
2. Add the antibody to a final concentration of 1:40 and mix.
3. Incubate for 60 minutes at 4°C.
4. Centrifuge to pellet the cells and discard the supernatant.
5. If using thymocytes or lymph node cells, resuspend to the original volume in Low-Tox—M® rabbit complement diluted 1:10 in cytotoxicity medium. If using splenocytes or bone marrow cells, resuspend to the original volume in baby rabbit complement diluted 1:10 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 vs dead cells in a hemaytometer. Cytotoxic index (C.I) can be calculated as shown in **FIGURE 1**.

Antibody Titration:
Cell Source: Spleen cells
Donors: BALB/c
Cell Concentration: 1 x 10e6 cells/ml
Complement: Baby Rabbit Complement
Complement Concentration: 1:10
see **FIGURE 2**

Strain Distribution:
Antibody Concentration: 1:20
Strains Tested: see **FIGURE 3**

Tissue Distribution:
Antibody Concentration: 1:40
Strain: Balb/c

Cell Source - C.I.
Thymus: 4
Spleen: 46
Lymph Node: 22
Bone Marrow: 17

NOTE: This antibody is not recommended in flow cytometry for cell preparations containing B cells due to the possibility of cross reactivity between the secondary antibody and surface immunoglobulins.

NOTES:

1. 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 I the same problem. We screen for batches of BSA giving low background in the presence of complement and use the selected BSA for preparing Cytotoxicity Medium®.
2. 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 wil 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. Low-Tox-M® rabbit complement is absorbed to remove toxicity to mouse lymphocytes, while maintaining its high complement activity. When used in conjunction with cytotoxicity medium®, this reagent provides a highly potent source of complement with minimal background toxicity.
4. Baby rabbit complement provides high complement activity with low background toxicity.

Product images:

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

Figure 3.

Strain	Haplotype	+/-
A.TH	H-2K^{s}D^{d}	-
AKR/J	H-2^{k}	-
C3H/He	H-2^{k}	-
C57BL/6	H-2^{b}	-
A.TL	H-2K^{s}D^{d}	-

Figure 2: Reciprocal of Antibody Dilution (K = thousand)

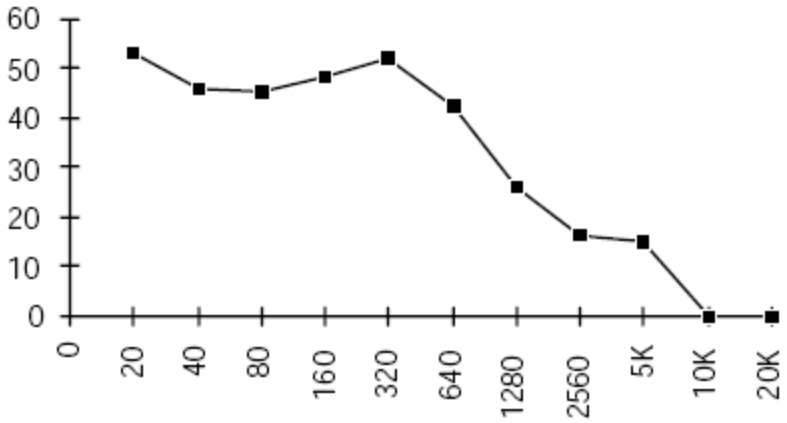

Figure 1: Cytotoxic Index (C.I).