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CL071 Monoclonal Antibody to MHC Class II I-Ak Ek - Ig Fraction

Quantity: 1 ml

Host / Isotype: Mouse / IgG2a

Clone: A303

Immunogen: Recipient: A.TH
Donor: A.TL
Fusion Partner: Spleen from immunized recipient fused with myeloma NSI-1-Ag4-1

Format: **State:** Lyophilized Ig fraction of Ascites
Buffer System: PBS
Reconstitution: Restore with 1.0 ml of distilled water.

Specificity: This monoclonal antibody defines the antigen specificity Ia.m48. This antigen is a combinatorial Ia antigen generated by the complementation of genes in the I-Ak and I-Ek subregions. The monoclonal antibody recognizes this combinatorial antigen on the Ak:Ek molecule. This monoclonal antibody is directly cytotoxic in the presence of complement. Only cells from those strains carrying both the I-Ak and I-Ek alleles are reactive. Recombinant strains carrying either the I-Ak or the I-Ek allele, are not reactive.
This antibody is reactive with Ia antigen bearing cells from strains carrying both the I-Ak and the I-Ek alleles but not with cells from recombinant strains carrying either the I-Ak and the I-Ek allele but not both. Independent haplotypes which display positive reactivity with this antibody are H-2k and also H-2P. Independent haplotypes which do not react include: H-2b, H-2d, H-2f, H-2u, H-2s and H-2r. See reference for full discussion.
Species: Mouse.
Other species not tested.

Add. Information: 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 0.22 µ Millipore filter (or equivalent).

Storage: Prior to reconstitution store at -20°C.
Following reconstitution store the antibody at -70°C.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General Readings: 1. Harris, J.F. and Delovitch, T.L. 1980. Derivation of a monoclonal antibody which detects an Ia antigen encoded by two complementing I-subregions. J. Immunol. 125:2167.

Protocols: <u>RECOMMENDED METHOD FOR DEPLETING A CELL POPULATION OF I-AkEk ANTIGEN BEARING LYMPHOCYTES:</u>
1. Prepare a cell suspension from the appropriate tissue in Cytotoxicity Medium1 or equivalent. Remove red cells (where necessary) by purification of viable lymphocytes on Lympholyte-M density cell separation medium. After washing, adjust the cell

concentration to 1x10e7 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 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 Low-Tox-M Rabbit Complement, 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 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 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 with 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. Alternately, 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 I-AkEk ANTIGEN BEARING CELLS IN A POPULATION:

1. Prepare a cell suspension from the appropriate tissue in Cytotoxicity Medium1 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 cell concentration to 1x10e6 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. Resuspend to the original volume in Low-Tox-M Rabbit Complement3 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 vs dead cells in a hemacytometer. Cytotoxic index (C.I.) can be calculated as shown in **FIGURE 1**.

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 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 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 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. Low-Tox-MRabbit Complement is absorbed to remove toxicity to mouse ymphocytes, 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.

Antibody Titration:
Cell Source: A.TL (I-AkEk) enriched splenic B cells
A.TH (I-AsEs) enriched splenic B cells
Cell Concentration: 1x10e6 cells per ml.
Complement: Low-Tox-M Rabbit Complement
Complement Concentration: 1:12

Procedure:
Two stage cytotoxicity as described in Recommended Method for Determining Percent of I-AkEk Antigen Bearing Cells in a Population.

STRAIN DISTRIBUTION:
Procedure: As above
Antibody concentration: 1:20
Strains tested: see **FIGURE 3**

Pictures:

Figure 3

Strain	Haplotype	+/-
A.TL	$I\text{-}A^kE^k$	+
BDP	$I\text{-}A^pE^p$	+
A.TH	$I\text{-}A^sE^s$	-
B10.A(4R)	$I\text{-}A^kE^b$	-
B10.HTT	$I\text{-}A^sE^k$	-

Figure 2: Enriched splenic B-cells were prepared by treatment of Lympholyte-M purified spleen lymphocytes with Monoclonal Anti-Thy-1.2 Antibody plus complement, followed by removal of dead cells on Lympholyte-M .

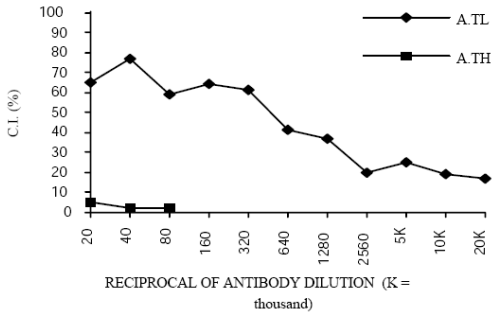

Figure 1: Cytotoxic index

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