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SM083 Monoclonal Antibody to MHC Class II I-Ak - Ig Fraction

Alternate names: H-2 class II histocompatibility antigen A-K alpha chain, H2-Aa

Quantity: 1 ml

Uniprot ID: P01910

NCBI: 10090

Host / Isotype: Mouse / IgG2a

Clone: 14V.18

Immunogen:
A.TL spleen.
Donor: A.TH spleen.
Fusion Partner: P3-X63-Ag8

Format:
State: Lyophilized Ascitic fluid 0.8 µ filtered (non-sterile)
Reconstitution: Restore with 1.0 ml of cold distilled water.

Applications:
Flow Cytometry.
Cytotoxicity Analysis (See Protocols).
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity:
This Monoclonal I-A^k antibody is a cytotoxic antibody specific for cells expressing the Ia antigen coded for by the A subregion of the k haplotype. The reaction pattern of this antibody with a panel of inbred and recombinant haplotypes demonstrates that the antibody reacts with Ia.m2, a private specificity of the H-2^k haplotype.
This antibody can be used to quantitate or to eliminate cells bearing the I-A^k (Ia.m2) antigen.
Species: Mouse.
Other species not tested.

Storage:
Store lyophilized at 2-8°C for 6 months or at -20°C long term.
After reconstitution store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C long term.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

Protocols:
CYTOTOXICITY ANALYSIS:
Method:
1. Prepare a cell suspension from the appropriate tissue in Cytotoxicity Medium (a) or equivalent. Remove red cells and dead cells (where necessary) by purification of viable lymphocytes on Lympholyte®-M (b) 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 1:80 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 Low-Tox®-M Rabbit Complement (c) diluted to the recommended concentration in Cytotoxicity Medium.
6. Incubate for 60 minutes at 37°C.

For research and in vitro use only. Not for diagnostic or therapeutic work.
Material Safety Datasheets are available at www.acris-antibodies.com or on request.

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 Shown in Figure 1.
C.I = %cyt (antibody+complement) - %cyt (complement alone) x 100
100% - %cyt (complement above)

Results:
Antibody Titration by Cytotoxicity Analysis:
Cell Source: Enriched splenic B cells.
Donor: A.TL
Cell Concentration: 1.1x106 cells/ml.
Complement: Low-Tox®-M Rabbit Complement.
Complement Concentration: 1:12
Procedure: Two-stage cytotoxicity.

Tissue Distribution by Cytotoxicity Analysis:
Antibody Concentration Used: 1:40
Strain: C3H/He
Cell Source: C.I.
Thymus: 0
Spleen: 54
Lymph Node: 35
Enriched B-cells: 70

Strain Distribution by Cytotoxicity Analysis:
Antibody Concentration Used: 1:40

CYTOTOXICITY DEPLETION ASSAY:
Method:
1. Prepare a cell suspension from the appropriate tissue in Cytotoxicity Medium (a) or equivalent. Remove red cells and dead cells (where necessary) by purification of viable lymphocytes on Lympholyte®-M 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 1:40 and mix. Alternatively, pellet the cells and resuspend in antibody diluted 1:40 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 Complementn (c) , 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 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.

FUNCTIONAL TESTING:

Method:

Cells were treated as described in **Cytotoxicity Depletion Assay**.
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.

Results:

Cell Source: Splenocytes.
Donors: C3H/He and C57BL/6
Cell Concentration: 1x10e7 cells/ml
Antibody Concentration Used: 1:40
Complement: Low-Tox®-M Rabbit Complement
Complement Concentration Used: 1:10
Treatment of C3H/He splenocytes with SM083 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 C57BL/6 cells had no effect on either plaque-forming cell number or cytotoxic T cell function. These results are consistent with the removal of I-Ak bearing cells and their related activities.

NOTES:

a. 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 backg round killing in the presence of 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.
b. Lympholyte®-M cell separation medium is a 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. 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.

Pictures:

Figure 3.

	Haplotypes								
Strains	K	A	B	J	E	C	S	G	D	+/-
A.TH	s	s	s	s	s	s	s	s	d	-
A.TL	s	k	k	k	k	k	k	k	d	+
B10.A	k	k	k	k	k	d	d	d	d	+
B10.A (4R)	k	k	b	b	b	b	b	b	b	+
B10.BR	k	k	k	k	k	k	k	k	k	+
B10.D2	d	d	d	d	d	d	d	d	d	-
BALB/c	d	d	d	d	d	d	d	d	d	-
C57BL/6	b	b	b	b	b	b	b	b	b	-

Figure 2.