OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850
UNITED STATES
Phone: +1-888-267-4436
Fax: +1-301-340-8606
techsupport@origene.com

OriGene Technologies GmbH
Schillerstr. 5
32052 Herford
GERMANY
Phone: +49-5221-34606-0
Fax: +49-5221-34606-11
info-de@origene.com

CL065 Polyclonal Antibody to MHC Class II Ia (reacts with all haplotypes except H-2s) - Alloantiserum

Quantity: 1 ml

Host: Mouse

Immunogen: A.TH mice with A.TL splenocytes

Format: **State:** Lyophilized Serum
Reconstitution: Restore with 1 ml of cold distilled water.

Applications: As a cytotoxic antibody, it can be used with complement for the enumeration or elimination of B cells, and is particularly well-suited as a positive control serum for B cell typing. The antiserum can also be used for biochemical studies of HLA-DR molecules.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: Mouse Ia alloantiserum is a broadly reactive Ia^k antiserum. The antiserum is prepared by immunizing A.TH mice with A.TL splenocytes. These two strains are congenic, differing at the I region of the H-2 complex, but identical at the K and D regions. The two strains are characterized by the following H-2 haplotypes:

	I							
	K	A	B	E	C	S	G	D
A.TH	s	s	s	s	s	s	s	d (K^s I^s D^d)
A.TL	s	k	k	k	k	k	k	d (K^s I^k D^d)

The I region gene products (Ia antigens) potentially detected by this antiserum, are those controlled by the following subregions: A^k, B^k, E^k, C^k, i.e. it is a broadly reactive anti-Iak antiserum. It detects each of the following public and private specificities: Ia.1,2,3,7,15,19, and 22. As a result, this antiserum crossreacts with all standard haplotypes (i.e. H-$2^{b,d,k,p,q,r}$) but does not crossreact with H-2s. The Ia antigens are expressed as cell surface antigens on lymphocyte subpopulations and macrophages and perhaps other non-lymphocytic cells. Since the A.TH anti-A.TL antiserum is cytotoxic, treatment of immunologically competent cell populations with this antiserum plus complement can quantitate cells bearing the corresponding Ia antigen or eliminate these cells from the population for functional studies.
Ia Antigens on Human B Lymphocytes: A.TH anti-A.TL antiserum is strongly cytotoxic to human B lymphocytes but is not cytotoxic to human T-lymphocytes. This antiserum appears to recognize a determinant present on both mouse Ia and human HLA-DR antigens. The antiserum reacts with B lymphocytes of all individuals, although there is one report that B cells of some individuals are not reactive. In addition to human peripheral blood B lymphocytes, this antiserum reacts with chronic lymphatic leukemia (CLL) cells and at least a portion of monocytes.
Species: Mouse, Human.
Other species not tested.

Add. Information: This antiserum is not sold as sterile, but can be sterilized by filtration if necessary. To minimize loss of volume during filtration, dilute the antiserum to the final working concentration in the appropriate medium before filtration and filter through a 0.22 µm filter.

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.

General Readings:

1. David, C.S., (1976) Serologic and genetics aspects of murine Ia antigens. Transplant. Rev. 30, 299.
2. Hämmerling GJ. Tissue distribution of Ia antigens and their expression on lymphocyte subpopulations. Transplant Rev. 1976;30:64-82. PubMed PMID: 59979.
3. Shreffler, D.C., David, C.S., Cullen, S.E., Frelinger, J.A., and Niederhuber, J.B., (1976) Serological and functional evidence for further subdivision of the I region of the H-2 gene complex. Cold Spring Harbor Symp. Quant. Biol. 41, 477.
4. Delovitch, T.L., and Falk, J.A. (1979) Immunochemical evidence for structural homology between murine and human Ia antigens. Immunogenetics 8:405.
5. Ing, P.M., Falk, J.A. Letarte, M. Delovitch, T.L., and Falk, R.E., (1979) Serologic cross reactions of murine and human Ia antigens. Transplant. Proc. 11:1745.
6. Lunney JK, Mann DL, Sachs DH. Sharing Ia antigens between species. III. Ia specificities shared between mice and human beings. Scand J Immunol. 1979;10(5):403-13. PubMed PMID: 94693.

Protocols:

METHOD FOR USE WITH MOUSE LYMPHOCYTES:

RECOMMENDED METHOD FOR DEPLETING A MOUSE CELL POPULATION OF Ia ANTIGEN BEARING CELLS:

1. Prepare a cell suspension from the appropriate tissue (e.g. spleen, lymph node, etc.) in Cytotoxicity Medium[1] or equivalent. Remove erythrocytes and dead cells (where necessary) by purification on Lympholyte®-M density cell separation medium[2]. After washing, adjust the cell concentration to $1.1x10^7$ cells per ml in cytotoxicity medium.
2. Add the anti-Ia antiserum to a final concentration of 1:20.
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 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 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.

RECOMMENDED METHOD FOR DETERMINING PERCENT OF Ia ANTIGEN BEARING CELLS IN A MOUSE CELL POPULATION:

1. Prepare a cell suspension from the appropriate tissue in Cytotoxicity Medium or equivalent. Remove erythrocytes and dead cells (where necessary) by purification on Lympholyte®-M density cell separation medium[2]. After washing, adjust the cell concentration to $1.1x10^6$ cells per ml in cytotoxicity medium.
2. Add the anti-Ia antiserum to a final concentration of 1:60.
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 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 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:

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

NOTES:

1. 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.
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 erythrocytes cells and dead cells. The density of this medium is 1.087-1.089. 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®-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. Each batch of Low-Tox®-M Rabbit Complement is accompanied by a spec sheet providing recommended concentrations for use with anti-Ia antisera.

METHODS FOR USE WITH HUMAN LYMPHOCYTES:

RECOMMENDED METHOD FOR USE AS A POSITIVE CONTROL SERUM FOR HUMAN B

CELL TYPING:
Dilute the antiserum 1:15 - 1:20 in standard culture medium before addition to the microlymphocytotoxicity trays. The assay should then be carried out according to your standard protocol. If the trays are to be frozen and stored after addition of the diluted antiserum, dilution should be carried out in a medium containing serum (e.g. McCoy's 5a with 20% fetal bovine serum) or BSA (e.g. McCoy's 5a with 6% BSA).
Note 1: Low-Tox®-H Rabbit Complement provides an excellent source of non-toxic rabbit complement for B cell typing.
Note 2: For the detection or elimination of Ia positive human cells with this anti- Ia plus complement, optimum concentrations of antiserum and complement will depend on the source of cells, cell concentration, and on the particular system used. We therefore recommend that you titrate both antibody and complement in order to establish optimum parameters in your system.
Note 3: A.TH anti-A.TL antiserum reacts with at least a portion of human monocytes as well as B cells. This should be taken into consideration when determining percent of Ia positive cells in a mixed population, and when interpreting functional experiments.

SPECIFICATIONS:
Lot Number: 4954
Recipient Strain: A.TH
Donor Strain: A.TL
Immunizing Cells: Spleen lymphocytes

STRAIN DISTRIBUTION:
Procedure: as above
Antibody concentration: 1:80
Strains tested: See **Figure 3**

TISSUE DISTRIBUTION:
Procedure: as above
Target cells: A.TL spleen, lymph node, thymus and bone marrow lymphocytes
Cell concentration: $1.1x10^6$ cells per ml
Antibody concentration: 1:160
Low-Tox®-M Rabbit Complement: 1:25

Cell Source : C.I.
Thymus: 2
Spleen: 45
Lymph Node: 30
Bone Marrow: 3

FUNCTIONAL TESTING:
Cell Source: Splenocytes
Donors: C57BL/6 and C3H/He
Cell Concentration: $1x10^7$ cells per ml
Antibody Concentration: 1:40

Complement: Low-Tox®-M Rabbit Complement
Complement Concentration: 1:22

Procedure:
Cells were treated as described in Recommended Method for Depleting a Cell Population of Ia Antigen Bearing Cells. 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 and C3H/He cells with anti-Ia plus complement resulted in a marked reduction in the number of plaque-forming cells. The generation of cytotoxic T cells (treatment of sample before sensitization) was not affected. However, cytotoxic T cell effector function (sample treated after sensitization) was partially inhibited. These results are consistent with the removal of Ia-bearing cells and their related activities.

ANTIBODY TITRATION:
Cell Source: A.TL (Iak) enriched splenic B cells
Cell Concentration: 1.1x10^6 cells per ml
Complement: Low-Tox®-M Rabbit Complement
Complement Concentration: 1:25

Procedure:
Two stage cytotoxicity as described in Recommended Method for Determining Percent of Ia Antigen Bearing Cells in a Mouse Cell Population.

Pictures:

Enriched splenic B cells 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.