OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850, US
Phone: +1-888-267-4436
https://www.origene.com
techsupport@origene.com
EU: info-de@origene.com
CN: techsupport@origene.cn

Product datasheet for CL060AP

MHC Class I H-2Ld Mouse Monoclonal Antibody [Clone ID: 30-5-7S]

Product data:

Product Type:	Primary Antibodies
Clone Name:	30-5-7S
Applications:	CT
Recommended Dilution:	**Cytotoxicity Analysis** (See Protocol).
Reactivity:	Human
Host:	Mouse
Isotype:	IgG2a
Clonality:	Monoclonal
Immunogen:	Recipient: BALB/c-H-2dm2 Donor: BALB/c Spleen cells Fusion Partner: SP2/0.Ag14
Specificity:	This Monoclonal antibody detects the public specificity H-2.65 of the H-2L^{d} antigen. This antibody also recognizes H-2D^{q} and H-2L^{q} molecules.
Formulation:	PBS State: Aff - Purified State: Liquid purified IgG fraction Preservative: 0.02% Sodium Azide
Purification:	Affinity Chromatography on Protein G
Conjugation:	Unconjugated
Storage:	Store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Database Link:	P01897
Synonyms:	H2-L
Note:	Protocol: **RECOMMENDED METHOD FOR DEPLETING A CELL POPULATION OF H-2L^{d} BEARING LYMPHOCYTES:** 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®[2] cell separation medium. After washing, adjust cell concentration to 1 x 10^7 cells per ml in cytotoxicity medium.

2. Add the antibody to a final concentration of 1:25 and mix. Alternatively, pellet the cells and resuspend in antibody diluted 1:25 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, it is recommended that the cells be resuspended to the original volume in Low-Tox-M® Rabbit Complement[3] diluted 1:10 in cytotoxicity medium. If using splenocytes or bone marrow cells, it is recommended that the cells be resuspended to the original volume in Baby Rabbit Complement 4 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 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 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 will 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 S*tep.6*, provided that the dead cells will not interfere with subsequent assays.

RECOMMENDED METHOD FOR DETERMINING PERCENT OF H-2L^d 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 cell concentration to 1 x 10^6 cells per ml in cytotoxicity medium.
2. Add the antibody to a final concentration of 1:50 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, it is recommended that the cells be resuspended to the original volume in Low-Tox-M® Rabbit Complement diluted 1:10 in cytotoxicity medium. If using splenocytes or bone marrow cells, it is recommended that the cells be resuspended 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 hemacytometer. The Cytotoxic Index (C.I) can be

calculated as follows:

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

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-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.

ANTIBODY TITRATION:

See **Figure 1.**
Cell Source: Splenocytes
Donors: BALB/c
Cell Concentration: 1×10^6 cells/ml
Complement: Low Tox®-M Rabbit Complement
Complement Concentration: 1:10
Procedure: Two stage cytotoxicity

Tissue Distribution:

Antibody Concentration: 1:40
Strain: BALB/c

Cell Source - C.I.

Thymus: 55

Spleen: 95
Lymph Node: 98
Bone Marrow: 54

Strain Distribution:
Antibody Concentration: 1:20
Strains Tested:

Strain	Haplotype	+/-
BALB/c	$H\text{-}2^{d}$	+
A.TH	$H\text{-}2K^{k}D^{d}$	+
A.TL	$H\text{-}2K^{k}D^{d}$	+
B.10A(3R)	$H\text{-}2K^{b}D^{d}$	+
C3H/He	$H\text{-}2^{k}$	-
C57BL/6	$H\text{-}2^{b}$	-

Functional Testing:
Cell Source: Splenocytes
Donors: BALB/c and C3H/He
Cell Concentration: 1×10^{7} cells/ml
Antibody Concentration: 1:25
Complement: Baby Rabbit Complement
Complement Concentration: 1:10

Procedure:
Cells were treated as described in Recommended Method for Depleting a Cell Population of H-2Ld 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 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 BALB/c splenocytes with anti-$H\text{-}2L^{d}$ plus complement resulted in a marked reduction in the number of plaque-forming cells and essentially eliminated cytotoxic T cells, as assessed by Jerne haemolytic plaque assay and CTL assay, respectively. Treatment of C3H/He splenocytes had no effect. These resultra are consistent with the depletion of H-2Ld bearing cells.

Product images:

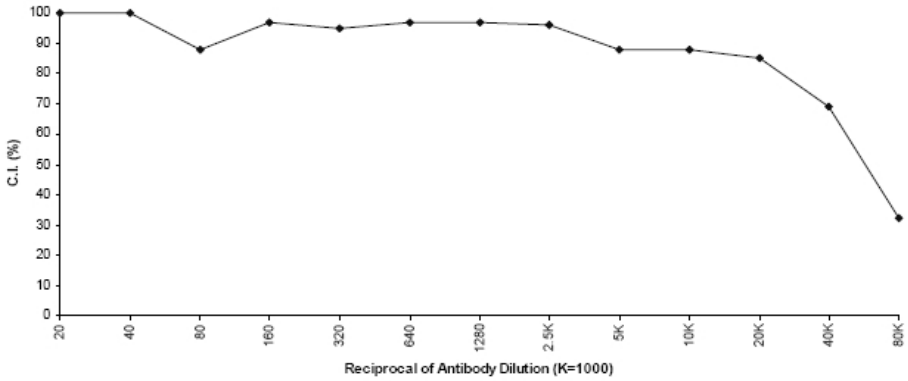

Figure 1. Antibody Titration