Mouse anti-Mouse H-2KbDb (private) Monoclonal Antibody

Anti-Mouse H-2KbDb (private), Purified (Clone 5041.16.1) (mouse IgG2a)

CATALOG #	HOST	REACTIVITY	APPLICATIONS
AAA74179	**Mouse**	**Mouse**	**Cytotoxicity Assay, Immunohistochemistry**

PRODUCT SYNONYMS

H-2KbDb (private); Monoclonal Antibody; Anti-Mouse H-2KbDb (private); Purified (Clone 5041.16.1) (mouse IgG2a); Purified Anti-Mouse H-2KbDb Monoclonal Antibody; anti-H-2KbDb (private) antibody

PRODUCT SPECIFICATIONS

Host	Mouse
Reactivity	Mouse
Clonality	Monoclonal
Isotype	Mouse IgG2a
Clone Number	5041.16.1
Specificity	H-2KbDb (Mouse H-2KbDb)
Purity/Purification	Purified from ascitic fluid via protein G affinity chromatography.
Form/Format	250 ug purified IgG buffered in PBS + 0.02% NaN3.
Concentration	1 mg/ml (varies by lot)
Applicable Applications for anti-H-2KbDb (private) antibody	CA (Cytotoxic Assay), IHC (Immunohistochemistry)
Donor	bm12 (thymus, lymph node and spleen cells)
Fusion Partner	NS-1
Recipient	CBA/J
Strain Distributions	H-2Kb and/or H-2Db positive strains

RECOMMENDED METHOD FOR DEPLETING A CELL POPULATION OF H- 2Kb AND/OR H-2Db BEARING LYMPHOCYTES	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 cellseparation medium. After washing, adjust the cell concentration to 1x107 cells per mlin Cytotoxicity Medium. 2 Add the antibody to a final concentration of 1:100 and mix. Alternatively, pellet thecells and resuspend in antibody diluted 1:100 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 1:10 with medium, and add 1/10 volume of 1% Trypan Blue. After 3-5 minutes, score live versus dead cells in a haemacytometer. 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 treated cell suspensions over an equal volume of Lympholyte® -M cell suspension 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 H-2Kb AND/ OR H-2Db 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 1x106 cells per ml in Cytotoxicity Medium. 2. Add the antibody to a final concentration of 1:1000 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 ytotoxicity 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 versus ead cells in a haemacytometer. Cytotoxic Index (C.I.) can be calculated as follows: C.I. = 100 x %cyt. (antibody + complement) - yt. (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 he 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 ells ot 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, abbit 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.
Preparation and Storage	Stable at 4° C. For long term storage, aliquot and freeze unused portions at -20°C in volumes appropriate for single usage. Avoid repeated freeze/thaw cycles

IMAGES

Application Data

Application Data

ADDITIONAL INFORMATION

Related Product Information for anti-H-2KbDb (private) antibody	anti-mouse H-2KbDb monoclonal antibody is specific for cells expressing the H-2K antigen coded for by the b haplotype and for cells expressing the H-2D antigen coded for by the b haplotype

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Small volumes of product may become entrapped in the seal of the vial during shipment and storage; if necessary, briefly centrifuge the vial on a tabletop centrifuge to dislodge any liquid in the cap.

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Online datasheet: https://stage.aaabiotech.com/monoclonal/h-2kbdb-private-antibody-clone-5041-16-1-cytotoxic-assay-immunohistochemistry-elisa/74179