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Product datasheet for CL090

MHC Class II I-Ad Mouse Monoclonal Antibody [Clone ID: 34-5-3S]

Product data:

Product Type:	Primary Antibodies
Clone Name:	34-5-3S
Applications:	CT
Recommended Dilution:	**Flow Cytometry.**
Reactivity:	Mouse
Host:	Mouse
Isotype:	IgG2a
Clonality:	Monoclonal
Immunogen:	BDF splenocytes Donor: C3H splenocytes Fusion Partner: SP2/0-Ag14
Specificity:	This monoclonal antibody specific for cells expressing the Ia antigen coded for by the A subregion of the d, b, p, and q haplotypes. (ie. I-Ad,b,p,q).
Formulation:	State: Ascites State: Lyophilized Ascites filtered to 0.45 mm
Reconstitution Method:	Restore with 0.5 ml of cold distilled water.
Conjugation:	Unconjugated
Storage:	Prior to and following reconstitution store the antibody at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Note:	Protocol: **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.

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4. Centrifuge to pellet the cells and discard the supernatant.
5. Resuspend to the original volume in Low-Tox®-M Rabbit Complementc 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 versus dead cells in a hemacytometer. Cytotoxic Index (C.I.) can be calculated as shown in **Figure 1.**

RESULTS - ANTIBODY TITRATION BY CYTOTOXICITY ANALYSIS:
see FIGURE 2
Cell Source: Splenocytes
Donor: DBA
Cell Concentration: 1.1 x 10e6 cells/ml
Complement: Low-Tox-M Rabbit Complement
Complement Concentration: 1:10

TISSUE DISTRIBUTION BY CYTOTOXICITY ANALYSIS:
Antibody Concentration used: 1:40
Strain: DBA

Cell Source:
Thymus: 0%
Spleen: 54%
Lymph Node: 16%
Bone Marrow: 12%

STRAIN DISTRIBUTION BY CYTOTOXICITY ANALYSIS:
Antibody Concentration Used: 1:40
Strains Tested: C57BL/6; C3H/He; DBA; A.TH; A.TL
Cells Killed by Treatment: C57BL/6; DBA
Cells Not Killed by Treatment: C3H/He, A.TH; A.TL

CYTOTOXIC DEPLETION ASSAY:
1. Prepare a cell suspension from the appropriate tissue (e.g. spleen, lymph node, etc.) in Cytotoxicity Mediuma or equivalent. Remove erythrocytes and dead cells (where necessary) by purification on Lympholyte-M density cell separation mediumb. After washing, adjust the cell concentration to 1.1x10e7 cells per ml in cytotoxicity medium.
2. Add the antibody to a final concentration of 1:40.
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 Complement3,4.

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.

FUNCTIONAL ANALYSIS:

Method:
Cells were treated as described in Cytotoxic 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. In vitro immunizations were used in all experiments.

Results:
Cell Source: Splenocytes
Donors: C3H/He and BALB/c
Cell Concentration: 1x10e7 cells/ml
Antibody Concentration: 1:100
Complement: Low-Tox®-M Rabbit Complement
Complement Concentration: 1:10
Treatment of BALB/c splenocytes with CL8713A 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 C3H/He cells had no effect on either plaque forming cell number or cytotoxic T cell function. These results are consistent with the removal of I-Ad 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 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.
b. 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.
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.

Product images:

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

Figure 1.

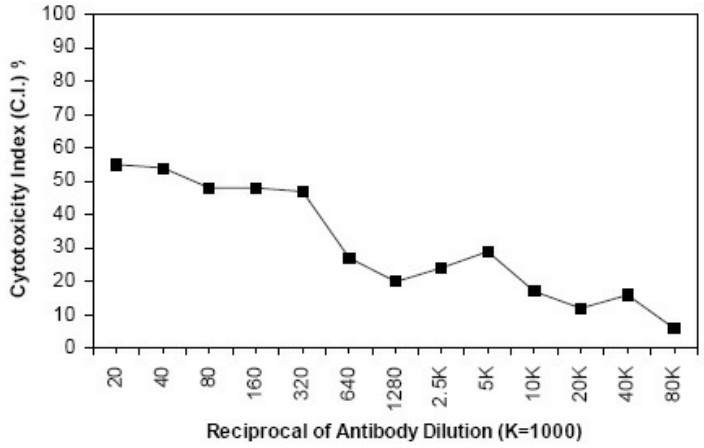

Figure 2.