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Product datasheet for CL007A

Cd8a Rat Monoclonal Antibody [Clone ID: YTS169.4]

Product data:

Product Type:	Primary Antibodies
Clone Name:	YTS169.4
Applications:	CT, FC, IHC
Recommended Dilution:	- Flow Cytometry Analysis (see Protocols). - Cytotoxicity Analysis (see Protocols). - Functional Analysis (see Protocols). - Cytotoxic Depletion of CD8a (Ly 2) positive Lymphocytes (see Protocols). - IN VIVO depletion of CD8a (Ly 2) positive Lymphocytes by opsonization for adoptive transfer (see Protocols). - Long term IN VIVO depletion of CD8a (Ly 2) positive Lymphocytes by direct Intra-Administration (see Protocols). - (Reported to be useful in immunohistochemistry on frozen sections.)
Reactivity:	Mouse
Host:	Rat
Isotype:	IgG2b
Clonality:	Monoclonal
Immunogen:	Mouse Ly-2 thymocytes. **Donor**: (Lou x DA) F1 rat. **Fusion Partner:** myeloma Y3/Ag1.2.3.
Specificity:	This purified anti-CD8a (Ly 2) monoclonal antibody reacts with a protein of approximately 30 kDa found on mouse thymocytes and mouse cytotoxic/ suppressor T cells. It does not bind to mouse helper/inducer T cells. It binds to T lymphocytes from all mouse strains regardless of phenotypic expression (i.e. reacts with T lymphocytes from mouse strains expressing the Ly 2.1 or Ly 2.2 phenotype). It can be used to investigate the role of T cells in models for infectious disease, autoimmunity, transplantation tolerance and fundamental aspects of immunology (Ref.1). It can also be useful to identify/eliminate cytotoxic or suppressor T lymphocytes in vivo or in vitro.
Formulation:	PBS, no preservative, 0.2 µm filtered State: Azide Free State: Liquid purified IgG fraction

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Concentration: lot specific

Conjugation: Unconjugated

Storage: Store 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.

Gene Name: CD8 antigen, alpha chain

Database Link: Entrez Gene 12525 Mouse
P01731

Background: The CD8 antigen is a cell surface glycoprotein found on most cytotoxic T lymphocytes that mediates efficient cell to cell interactions within the immune system. The CD8 antigen, acting as a coreceptor, and the T cell receptor on the T lymphocyte recognize antigen displayed by an antigen presenting cell (APC) in the context of class I MHC molecules. The functional coreceptor is either a homodimer composed of two alpha chains, or a heterodimer composed of one alpha and one beta chain. Both alpha and beta chains share significant homology to immunoglobulin variable light chains.

Synonyms: CD8 alpha chain, CD8A, MAL

Note: Protocol: **FLOW CYTOMETRY ANALYSIS:**

Method:
1. Prepare a cell suspension in media A. For cell preparations, deplete the red blood cell population with Lympholyte®-M cell separation medium.
2. Wash 2 times.
3. Resuspend the cells to a concentration of 2x10e7 cells/ml in media A. Add 50µl of this suspension to each tube (each tube will then contain 1x10e6 cells, representing 1 test).
4. To each tube, add 0.2 µg per 1 x 10e6 cells of this Ab.
5. Vortex the tubes to ensure thorough mixing of antibody and cells.
6. Incubate the tubes for 30 minutes at 4°C.
7. Wash 2 times at 4°C.
8. Add 100 µl of secondary antibody (FITC Goat anti-rat IgG (H+L)) at 1:500 dilution.
9. Incubate the tubes at 4°C for 30-60 minutes. (It is recommended that the tubes are protected from light since most fluorochromes are light sensitive).
10. Wash 2 times at 4°C in media B.
11. Resuspend the cell pellet in 50 µl ice cold media B.
12. Transfer to suitable tubes for flow cytometric analysis containing 15 µl of propidium iodide at 0.5 mg/ml in PBS. This stains dead cells by intercalating in DNA.

Media:
A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 µl of 2M sodium azide in 100 mls).
B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 µl of

2M sodium azide in 100 mls).

Results - Tissue Distribution by Flow Cytometry Analysis
Mouse Strain: BALB/c
Cell Concentration: 1x10e6 cells per tests
Antibody Concentration Used: 1.0 µg/10e6 cells
Isotypic Control: Rat IgG2b,k
Cell Source Thymus: 87.1% cells stained above control
Cell Source Splenic T cells: 29.75% cells stained above control

CYTOTOXICITY ANALYSIS:

Method:
1. Prepare a cell suspension from the appropriate tissue in Cytotoxicity Mediuma or equivalent. Remove red cells and dead cells (where necessary) by purification of viable lymphocytes on Lympholyte®-Mb 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 1:160 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 Complementc diluted 1:18 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 versus dead cells in a hemacytometer.

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.

Results - Antibody Titration
Cell Source: Thymocytes
Donor: C3H/He
Cell Concentration: 1.1x10e6 cells/ml
Complement: Low-Tox®-M Rabbit Complement
Complement Concentration: 1:18
Procedure: Two-stage cytotoxicity

Results - Tissue Distribution
Antibody Concentration: 1:160
Strain: C3H/He

Results - Strain Distribution
Antibody Concentration Used: 0.2 µg/10e6 cells
Strains Tested: C57BL/6, BALB/c, AKR/J, C3H/He
Positive: C57BL/6, BALB/c, AKR/J, C3H/He
Negative: none

FUNCTIONAL ANALYSIS:

Method:
Cells were treated as described in Cytotoxic Depletion CD8a (Ly 2) Positive Lymphocytes. 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 Concentration: 1x10e7 cells/ml
Antibody Concentration: 1:40
Complement: Low-Tox®-M Rabbit Complement
Complement Concentration: 1:18
Treatment of C3H/He splenocytes with anti-CD8a plus complement had no effect on the number of plaque-forming cells. However, marked inhibition of cytotoxic T cell function was found to occur when cells were treated before or after sensitization in the CTL assay. These results are consistent with the depletion of cytotoxic T cells of the Ly 2 phenotype (CD8a cells).

CYTOTOXIC DEPLETION OF CD8a (Ly 2) POSITIVE 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 lymphocytes on Lympholyte®-M density 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 1:160 and mix. Alternatively, pellet the cells and resuspend in antibody diluted 1:160 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 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 with 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.

IN VIVO DEPLETION OF CD8a (Ly 2) POSITIVE LYMPHOCYTES BY OPSONIZATION FOR ADOPTIVE TRANSFER:

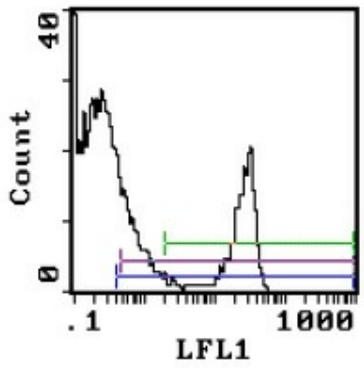
1. Dilute 1.0 ml of antibody to 1:50 for every 5x10e cells.
2. Incubate for 15 minutes before transfer to recipient animals (with or without washing away excess antibody).

LONG TERM IN VIVO DEPLETION OF CD8a (Ly 2) POSITIVE LYMPHOCYTES BY DIRECT INTRA-ADMINISTRATION:

1. Adult thymectomy of mice 5-6 weeks of age.
2. Two intra-venous (tail vein) injections of monoclonal antibody at 4-7 days apart (0.2 ml of appropriate dilution 1:1 to 1:5).
3. Wait 10-14 days for antibody to clear in vivo.
4. Depletion can be checked by:
a) immunofluorescence/flow cytometric analysis
b) frozen sections of spleen stained by immunoperoxidase

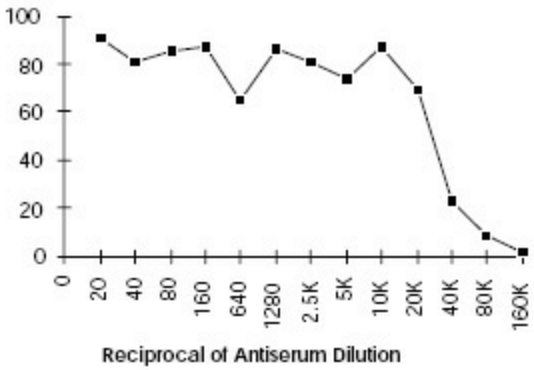
c) microcytotoxicity of peripheral blood samples

Product images:



Cell Source: Splenic T Cells
Percentage of cells stained above control: 19.1%

FLOW CYTOMETRY ANALYSIS - Tissue Distribution



CYTOTOXICITY ANALYSIS - Antibody Titration