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Product datasheet for AP31643SU-N

Lymphocytes Rabbit Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	CT
Recommended Dilution:	**Cytotoxicity studies** (See Protocols).
Reactivity:	Rat
Host:	Rabbit
Clonality:	Polyclonal
Immunogen:	Rat thymus, spleen and lymph node.
Specificity:	This antibody reacts with all Rat Lymphocytes and is strongly cytotoxic.
Formulation:	State: Serum State: Lyophilized Antiserum which has been adsorbed with Rat Erythrocytes and hepatocytes and filtered to 0.45µm (non-sterile).
Reconstitution Method:	Restore with 1.0 ml of distilled water.
Conjugation:	Unconjugated
Storage:	Store Lyophilized at -20°C. After reconstitution, aliquot and freeze unused portions at –70°C in volumes appropriate for single usage. Avoid repeated freeze/thaw cycles.
Stability:	Shelf life: one year from despatch.
Note:	Protocol: **Protein Concentration:** 271.5 +/- 16.6 mg/ml **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®-Rat[b] density 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 1:10 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®-R[c] diluted to the recommended concentration in Cytotoxicity Medium (~1:8).
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 follows:
C.I. = 100%Cyt (Ab+Complement) - %Cyt (Complement) / 100% - %Cyt (Complement).

Cytotoxicity Depletion Assay:

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®-Rat[b] 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 1:10 and mix. Alternatively, pellet the cells and resuspend in antibody diluted 1:10 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®-R Complement[c] diluted to the appropriate concentration in Cytotoxicity Medium.
(Recommended concentration included with each batch of Low-Tox®-R Complement.)
6. Incubate for 60 minutes at 37°C.
7. Monitor for percent cytotoxicity 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 with 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 a 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.

Results:
Cell Source: Thymus
Donor: Buffalo
Cell Concentration: 1x10e6 cells/ml
Complement: Low-Tox®-R Complement
Complement Concentration Used: 1:8

Procedure: Two-stage cytotoxicity as described below

Tissue Distribution By Cytotoxicity Analysis:
Procedure: See below
Strain: Fisher
Antibody Concentration Used: 1:10
Complement: Low-Tox®-R Complement
Complement Concentration Used: 1:8

Cell Source: C.I.%
Thymus: 100%
Spleen: 79%
Lymph Nodes: 87%
Bone Marrow: 86%

Functional Testing:

Method:
Cells were treated as described in**Cytotoxicity Depletion Assay**. Treated cells and controls were tested for:
a) mitogen proliferation assay: Concanavalin A (CON A), Phytohaemagglutinin (PHA) and Lipopolysaccharide (LPS).
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
Donor: Rat
Cell Concentration: 1x10e7 cells / ml.
Antibody Concentration: 1:20
Complement: Low-Tox®-R Complement at 1:8

a) Cell depletion with **AP31643SU-N** was found to inhibit the CON A, PHA and LPS responses (98%, 93% and 87% respectively).
b) Treatment of Rat splenocytes with **AP31643SU-N** plus complement essentially eliminated in vitro T effector cell function.

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 rat

and mouse lymphocytes, thus increasing the background killing in the presence of 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®-Rat cell separation medium is a density separation medium designed specifically for the isolation of viable rat lymphocytes. This separation medium provides a high and non-selective recovery of viable rat lymphocytes, removing red cells and dead cells. The density of this medium is 1.0940 +/- 0.001. Isolation of rat lymphocytes on cell separation medium of density 1.077 will result in high and selective loss of lymphocytes and should be avoided.

c. Low-Tox®-R complement is a highly active source of complement specifically designed for use with rat lymphocytes. When used in conjunction with Cytotoxicity Medium, this reagent provides a highly potent source of complement with minimal background cytotoxicity.

Product images: