OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850
UNITED STATES
Phone: +1-888-267-4436
Fax: +1-301-340-8606
techsupport@origene.com

OriGene Technologies GmbH
Schillerstr. 5
32052 Herford
GERMANY
Phone: +49-5221-34606-0
Fax: +49-5221-34606-11
info-de@origene.com

AP31645SU-L Polyclonal Antibody to Lymphocytes - Serum

Quantity: 5 ml

Host: Rabbit

Immunogen: Mouse thymus, spleen and lymph node cells.
Remarks: Immunizing Strain: BALB/c

Format: **State:** Lyophilized Antiserum which has been adsorbed with Mouse Erythrocytes and Hepatocytes.
Reconstitution: Restore with distilled water to initial volume.

Applications: **Cytotoxicity studies** (See Protocols).
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This antibody is strongly cytotoxic to all Mouse lymphocytes.

Species Reactivity: **Tested:** Mouse.

Add. Information: **Sterility:** This reagent is not sold as sterile, but can be sterilized by filtration if necessary. To minimize loss of volume during filtration, dilute to the final working concentration in the appropriate medium before filtration and filter through a 0.22 µm filter.
Heat Inactivation: 60 minutes at 56°C.
Absorption: Mouse erythrocytes/hepatocytes.

Storage: Store Lyophilized at 2-8°C or -20°C.
After reconstitution, aliquot and freeze unused portions at –70°C in volumes appropriate for single usage.
Avoid repeated freeze/thaw cycles.
Shelf life: one year from despatch.

Protocols: <u>Recommended Mehod for Depleting A Cell Population Of Mouse Lymphocytes:</u>

1. Prepare a cell suspension from the appropriate tissue in Cytotoxicity Medium (1) or equivalent.
Remove red cells and dead cells (where necessary) by purification of viable lymphocytes on Lympholyte®-M (2) density cell separation medium. After washing, adjust the cell concentration to 1x10e7 cells per ml in Cytotoxicity Medium.
2. Add the antiserum to a final concentration of 1:40 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 (3) Rabbit Complement, diluted to the appropriate concentration in Cytotoxicity Medium. (Recommended concentration included with each batch of Low-Tox®-M 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. Layering the suspension on cell separation medium and centrifuging at room temperature as per the instructions provided can do this. Live cells will form a layer at the interface, while 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 then 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.

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 cell 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 1.0865-1.0885. 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 murine 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.

Recommended Mehod for Determinig Percent Cytotoxicity With Anti-Mouse Lymphocytes Serum Plus Complement:
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 (2) density cell separation medium. After washing, adjust the cell concentration to 1.1 x10e6 cells per ml in Cytotoxicity Medium.
2. Add the antiserum to a final concentration of 1:40 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 Complement (3) diluted to the appropriate concentration in Cytotoxicity Medium. (Recommended concentration

included with each batch of Low-Tox®-M Rabbit Complement ~1:10 – 1:25).
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. = %Cyt (Ab+Complement) - %Cyt (Complement) / 100% - %Cyt (Complement).

Lot Specifications:
Antiserum Titration:
Cell Source: Thymus.
Donors: BALB/c.
Cell Concentration: 1.1x10e6 cells per ml
Complement: Low-Tox®-M Rabbit Complement (3)
Complement Concentration: 1:10
Procedure: Two stage cytotoxicity as described in **Recommended Method for Determining Percent Cytotoxicity with Anti-Mouse Lymphocyte Serum Plus Complement**.

Tissue Distribution:
Procedure: As above
Antiserum Concentration: 1:40
Strain: BALB/c
Cell Source (C.I.)
Thymus (98.0)
Spleen (80.0)
Lymph Node (95.0)
Bone Marrow (81.0)
Antiserum dilution that results in 50% cytotoxicity against thymus cells: ~ 1:1350.

Strain Distribution:
Target Cell: Thymus
Procedure: As above
Strains Tested (+/-)
C57BL/6 (+)
C3H/He (+)
ATH (+)
A.TL (+)
CBA (+)
BALB/c (+)

Functional Testing:
Cell Source: Splenocytes
Cell Concentration: 1x10e7 cells/ml.
Antiserum Concentration: 1:5

Complement: Low-Tox®-M at 1:10
Donors: BALB/c, C3H/He

Procedure:
Cells were treated as described in **Recommended Method for Depleting A Cell Population of Mouse Lymphocytes**. The remaining viable cells were exposed to the mitogens Concanavalin A (CON A), Phytohaemagglutinin (PHA), and Lipopolysaccharide (LPS). Cell depletion with Anti-Mouse Lymphocyte Serum was found to inhibit the CON A, PHA, and LPS responses.
Treatment of mouse splenocytes with Anti-Mouse Lymphocyte Serum plus complement essentially eliminated in vitro T effector cell function.

Pictures: