SZABO SCANDIC

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Produktinformation

- Forschungsprodukte & Biochemikalien
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- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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LOW-TOX®-M RABBIT COMPLEMENT

For Use with Mouse Lymphocytes

CL3051

DESCRIPTION:
Rabbit serum provides the most potent source of complement for cytotoxicity techniques utilizing antisera to mouse lymphocytes. However, rabbit serum is very toxic to mouse lymphocytes, particularly thymocytes. This toxicity can be reduced significantly by appropriate absorptions. Each lot is thoroughly tested for low toxicity and high activity and can be used without further processing.

PRESENTATION: 5x 1 ml, lyophilized.

STORAGE: Store at -20°C before reconstitution. After reconstitution, store at 4°C for a maximum of one hour.

RECONSTITUTION: Reconstitute with 1 ml of ice cold distilled water. Refrigerate the reconstituted material until used. Use as soon as possible after reconstitution. Discard the unused portion; storage of this reagent for long periods at 4°C or freezing and thawing of the reconstituted material will result in a marked reduction in potency.

NOTE:
1. The reconstituted material will appear cloudy. This is a result of the lyophilization process and does not affect potency.

2. Optimum concentration of this batch of Cedarlane Low-Tox®-M Rabbit Complement will depend on the system in which it is used. **This reagent should be titrated in your system to establish the concentration which provides maximum activity with an acceptable background toxicity**. See attached sheet for recommended concentrations.

STERILITY: This reagent is not sold as sterile. It can be sterilized by filtration if necessary. It is preferable to dilute the complement to a final working concentration before filtration, in order to minimize loss of volume.

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RECOMMENDED PROCEDURES FOR CYTOTOXICITY TECHNIQUES

A. Recommended Method for Determining Percent Cytotoxicity with Antibody Plus Complement

1. Prepare a cell suspension from the appropriate tissue in Cedarlane Cytotoxicity Medium[1] or equivalent. Remove red cells and dead cells (where necessary) by purification of viable lymphocytes on Cedarlane Lympholyte®-M[2] density cell separation medium. After washing, adjust the cell concentration to 1.2×10^6 cells per ml in Cytotoxicity Medium.

2. Add desired concentration of test antiserum and mix.

3. Incubate for 60 minutes at 4°C.

4. Centrifuge to pellet cells and discard the supernatant.

5. Resuspend to the original volume in Cedarlane Cytotoxicity Medium containing the appropriate concentration of Cedarlane 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 vs dead cells in a hemacytometer.
   Cytotoxic Index (C.I.) can be calculated as follows:

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

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B. Recommended Method for Depleting a Cell Population of a Subclass of Lymphocytes

1. Prepare a cell suspension from the appropriate tissue in Cedarlane Cytotoxicity Medium[1] or equivalent. Remove red cells and dead cells (where necessary) by purification of viable lymphocytes on Cedarlane Lympholyte®-M[2] cell separation medium. After washing adjust the cell concentration to 1 x 10^7 cells per ml in Cytotoxicity Medium.

2. Add desired concentration of appropriate antiserum 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 Cytotoxicity Medium containing the appropriate concentration of Cedarlane Low-Tox®-M Rabbit Complement (usually a dilution between 1:20 and 1:25).

6. Incubate for 60 minutes at 37°C.

7. Place on ice and monitor for % cytotoxicity before further processing. For this purpose, remove a small sample from each tube, dilute 1:10, and add 1/10th volume of 1% trypan blue. After 3 - 5 minutes, score live vs 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 treated cell suspension over an equal volume of Cedarlane Lympholyte®-M cell separation medium and centrifuging 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.

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LOW-TOX®-M RABBIT COMPLEMENT RECOMMENDED CONCENTRATIONS

Antiserum type	Recommended final dilution of rabbit complement	
	10^6 cells/ml	10^7 cells/ml
Rabbit anti-mouse T cell serum	1:20	1:15
Anti Thy 1 alloantisera	1:22	1:15
Anti Ly alloantisera	1:22	1:15
Anti-Ia alloantisera	1:20	1:10
Anti-H-2 alloantisera	1:20	1:10
Monoclonal antibodies to murine differentiation antigens	1:22	1:15
Monoclonal antibodies to murine class I antigens	1:20	1:10
Monoclonal antibodies to murine class II antigens	1:25	1:15

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NOTES:

1. Cedarlane 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 Cedarlane Cytotoxicity Medium.

2. Cedarlane Lympholyte®-M cell separation medium is a density separation medium designed specifically for the isolation of viable mouse lymphocytes. This separation medium provides a high and non-selective recovering of viable mouse lymphocytes, removing both red cells and dead cells. The density of the 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.

FOR RESEARCH ONLY

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CAUTION: the device contains material of human or animal origin and should be handled as a potential carrier and transmitter of disease.