PROTOCOL

AbSelect Mouse TCS Purification System

Applicable to: 832-0005 1 purification
832-0500 3 purifications

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INTRODUCTION

AbSelect Mouse resin has a very high affinity and specificity for mouse IgG molecules. The AbSelect Mouse TCS Purification System can be used to purify mouse IgG fractions from hybridoma supernatants. The binding strength of bovine IgG to the AbSelect Mouse resin is negligible; therefore, it can be used to selectively recover mouse IgG from TCS samples (see Appendices 1 and 2).

The method involves capture of the mouse antibody on the AbSelect Mouse resin and removal of unwanted substances using a simple wash procedure. The purified product is eluted and neutralized. The AbSelect Mouse TCS Purification System is not suitable for use with antibodies from other species.

The AbSelect Mouse TCS Purification System is fully compatible with both the Lightning-Link® and Lightning-Link® Rapid conjugation systems (available separately), which allow purified antibody to be labeled with a hands-on time of under 30 seconds.

KIT CONTENTS

- 1 or 3 bottles of AbSelect Mouse resin
- 1 bottle of 10x Binding Buffer
- 1 bottle of Wash Buffer
- 1 bottle of Elution Buffer
- 1 bottle of Neutralization Buffer
- 1 or 3 purification columns
- 1 or 3 concentrator spin columns

STORAGE AND COMPONENTS

The kit is shipped at ambient temperature. Store the kit at 4°C upon receipt.

AMOUNT OF MOUSE ANTIBODY THAT CAN BE PURIFIED

The mouse antibody to be purified should be in 10 to 25ml of tissue culture supernatant. Up to 1.5mg of antibody can be purified in each run.

STORAGE OF MOUSE ANTIBODY

Store at 4°C. Other storage conditions (e.g. frozen at -70oC) may also be satisfactory. The sensitivity of any particular mouse antibody to freeze-thaw should be determined by experimentation on small aliquots.

INSTRUCTIONS

1. Preparing the tissue culture supernatant

Add the 10x Binding Buffer to the tissue culture supernatant. The volume required is 1/10 of the volume of tissue culture supernatant. For example, to 20ml of tissue culture supernatant add 2ml of 10x Binding Buffer and mix by inversion.

2. Incubation of sample with resin

Add the AbSelect Mouse resin to the supernatant and incubate with mixing at room temperature for a minimum of 2 hours. Alternatively, incubate overnight at either 4°C or room temperature. Use the supernatant to rinse the bottle to recover all of the AbSelect Mouse resin.

3. Packing of the column

Support the column in an upright position and place a waste collection tube underneath (not provided).

Carefully transfer the supernatant-resin mix into the column. Sample volumes of more than 10ml will have to be added in aliquots. The AbSelect Mouse resin will collect in the bottom of the column. The unwanted supernatant will pass through the column and can be kept on ice until a successful outcome has been confirmed.

4. Wash procedure

Wash the column with the Wash Buffer to remove any non-bound protein. Place another waste collection tube (not provided) under the column and add 7ml of the Wash Buffer to the top of the column. Wait until it has all passed through and then repeat the wash procedure a total of three times.

Note: Wash the inner surface of the column to remove any residual starting material. Keep the wash fraction until successful outcome is confirmed.

5. Elution

See appendix 3 before carrying out this step.

The mouse antibody is eluted in 1ml fractions. Place a collecting tube under the column and add 1ml of Elution Buffer. Once all the buffer has passed through the column, remove the collection tube and add 0.25ml of Neutralization Buffer and mix. Repeat the elution process with a fresh collection tube three more times, each time neutralizing the sample as it is eluted. The Neutralization Buffer must be added as soon as possible to avoid prolonged exposure to low pH which can result in denaturation of the mouse IgG. The protein normally elutes in tubes 1 and 2 but you should confirm this using a test for protein (see Appendix 3) before pooling any of the tubes.

ANTIBODY CONCENTRATION (OPTIONAL)

If the concentration of the recovered mouse antibody is low then it can quickly and easily be concentrated using the antibody concentrator.

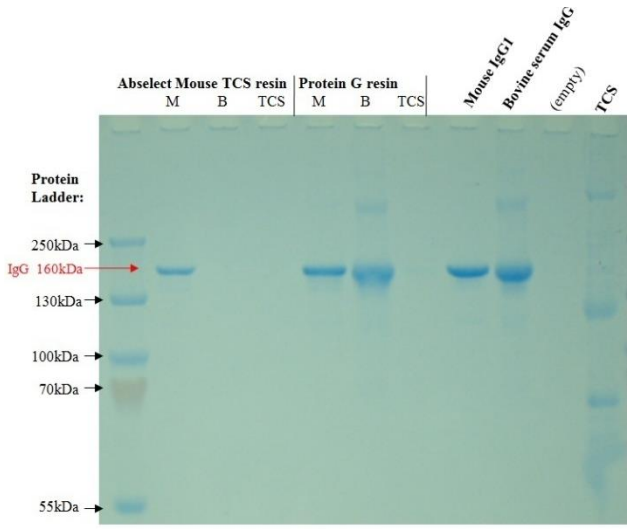
1. Add the mouse antibody to the top of the spin cartridge.
2. Spin for 1 to 3 minutes* in a microfuge at a recommended maximum speed of 15,000*g* to reduce the buffer volume in the spin cartridge to 50-100µl.
3. Repeat steps 1 and 2 as many times as is necessary to process the entire mouse antibody to the desired concentration. It may be necessary to discard any excess buffer collected in the collection tube between spins.
4. Recover the concentrated antibody from the top of the spin cartridge.

Note: It is advisable not to spin the mouse antibody dry as reconstitution of the antibody will be difficult and significant antibody loss and/or denaturation may occur.

*Spin times will vary depending on buffer composition and volume as well as centrifuge speed.

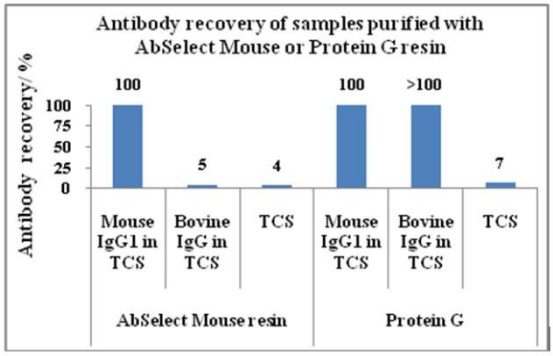
APPENDICES

Appendix 1: Gel electrophoresis of TCS samples purified with the AbSelect Mouse and Protein G resin



Legend: M = Mouse IgG1 in a TCS sample
B = Bovine serum IgG in a TCS sample
TCS = Tissue culture supernatant sample

Appendix 2: A representative bar graph comparing the effectiveness of AbSelect Mouse and Protein G resin



Appendix 3: Test for protein

Wherever possible protein values should be determined using absorbance at 280nm. An extinction co-efficient of 1.4 is generally used for IgG – so a 1mg/ml solution of IgG will give an absorbance value of 1.4 when measured with a 1cm path length.

Note: if a low volume/amount of antibody has been added, the concentration of protein in the eluates will be low. When other methods of determining IgG concentration are used such as BCA or Bradford protein assays, determinations should be performed before the addition of the Neutralization Buffer, as this can interfere with these reagents. Remove an aliquot for protein determination and neutralize the rest of the fraction immediately as the low pH of the elution buffer can denature the antibody.

When using Bradford-type reagents it is important to use an IgG standard curve. Failure to do this will result in incorrect antibody levels being calculated. If IgG is not available then a BSA standard curve can be used, but the IgG levels will be under-estimated by a factor of 2.3.

TECHNICAL SUPPORT

For technical enquiries get in touch with our technical support team at: technical.enquiries@expedeon.com

For further information see our website: www.expedeon.com