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AbSelect Mouse TCS Purification System (1 Purification, 10-25ml TCS)

Catalog No.: AC051-001

Quantity: 1 set

Reaction: 1 Purification, 10-25ml TCS

Kit Contents:

AC051-001-R:	AbSelect Mouse TCS resin
AC051-001-P:	1 Purification Column
AC051-001-B:	1 Bottle of 10x Binding Buffer
AC051-001-W:	1 Bottle of Wash Buffer
AC051-001-E:	1 bottle of Elution Buffer
AC051-001-N:	1 vial of Neutralizer
AC051-001-T1:	1 concentrator spin column and collection tube

Background:

AbSelect Mouse TCS resin has a high affinity for mouse IgG molecules. The AbSelect Mouse TCS Purification System can be used to purify mouse IgG fractions from hybridoma supernatants. The method involves capture of the antibody on the AbSelect Mouse TCS resin and the removal of unwanted substances using a simple wash procedure. The purified product is then eluted and neutralized. The

AbSelect Mouse TCS Purification System is not suitable for use with antibodies from other species. It should be noted that the binding strength of Bovine IgG to the AbSelect Mouse resin is negligible..

The AbSelect Mouse TCS Purification System **AC051** is fully compatible with both the Link-A-Light antibody labeling kits (available separately), which allow the purified antibody to be immediately labeled with a hands-on time of under 30 seconds.

Principle of the Procedure:

Step 1: Prepare the Tissue Culture Supernatant (TCS). **Step 2:** Transfer the AbSelect Mouse TCS resin to the prepared Supernatant and mix for 2 hours. **Step 3:** Transfer the solution into the column. **Step 4:** Wash the AbSelect Mouse TCS resin. **Step 5:** Elute and Neutralize purified antibody. **Step 6:** Confirm Elution using Protein Assay. **Step 7:** Concentrate the Mouse antibody (Optional).

Protocols:

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

2. Preparing the Tissue Culture Supernatant (TCS).
Add the 10x Binding Buffer **AC051-001-B** to the Tissue Culture Supernatant. The volume to add is 1/10 of the volume of Tissue Culture Supernatant. For example, for 20ml of Tissue Culture Supernatant add 2ml of 10x Binding Buffer **AC051-001-B** and mix by inversion.

3. Incubation of Sample with Resin

Add the AbSelect Mouse TCS Resin **AC051-001-R** to the Supernatant and incubate with mixing at room temperature for a minimum of 2 hours. Use the Supernatant to rinse the bottle to recover all of the AbSelect Mouse TCS Resin.

4. Packing of the column

Carefully pour the Supernatant-Resin mix into the column. Sample volumes of more than 10 ml will have to be added in aliquots. The AbSelect Mouse TCS 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.

5. Wash procedure

Wash the column with 7 ml of Washing Buffer **AC051-001-W** to remove any non-bound protein. Repeat the wash procedure three times.
Note: Wash the inner surface of the column to remove any residual starting material.

6. Elution

The Mouse antibody is eluted in 1ml fractions.
Place a collecting tube under the column and add 1ml of Elution Buffer (See Section 8).
Remove the collection tube and add 0.25 ml of Neutralizing Buffer **AC051-001-N**.
Cap the tube and place to one side.
Repeat the elution process three more times, each time neutralizing the sample as it is eluted.
The Neutralizing Buffer **AC051-001-N** 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 (Section 8) before pooling any of the tubes.

7. Antibody Concentration (Optional)

If the concentration of the recovered Mouse antibody is low then it can very quickly and easily be concentrated using our Antibody Concentration:

Step 1: Add the mouse antibody to the top of the spin cartridge.

Step 2: Spin for 1 to 3 minutes* in a microfuge at maximum speed to reduce the buffer volume in the spin cartridge to between 50 and 100 µl.

Step 3: Repeat steps 1 to 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.

Step 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 Mouse 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.

8. Test for Protein

Wherever possible protein values should be determined using absorbance at 280nm.

When other methods of are used such as BCA or Bradford protein assays, determinations should be performed before the addition of the Neutralization Buffer **AC051-001-N**, 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 protein 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.

Storage:

Storage of Kit: The kit is shipped at ambient temperature. Store the kit at 4°C upon receipt.

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