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AbSelect TCS Antibody Purification System (1 Purification, 10-50 ml TCS)

Catalog No.: AC049-001

Quantity: 1 set

Reaction: 1 Purification, 10-50 ml TCS

Kit Contents:

AC049-001-R:	AbSelect TCS Protein A Resin
AC049-001-P:	1 Purification Column
AC049-001-B:	1 Bottle of 10x Binding Buffer
AC049-001-W:	1 Bottle of Wash Buffer
AC049-001-E:	1 Bottle of Elution Buffer
AC049-001-N:	1 vial of Neutralization Buffer
AC049-001-T1:	1 Concentrator spin column and Collection tube

Background:

Antibodies are often generated from hybridoma cell lines and supplied in Tissue Culture Supernatant (TCS). TCS itself contains many substances that interfere with antibody labeling reactions. Successful labeling therefore requires antibodies to be purified prior to labeling. The AbSelect TCS system **AC049** is a fast and simple method to purify these antibodies.
AbSelect TCS is prepared by coupling highly purified Protein A to Agarose beads which can be used to purify IgG fractions from hybridoma supernatants. The method involves the capture of the antibody on the AbSelect TCS Resin and the removal of unwanted substances using a simple wash procedure. The purified product is then eluted and neutralized.
The components of the AbSelect TCS are fully compatible with the Link-A-Light antibody labeling kits (available separately), which allows the purified antibody to be immediately labeled with an Enzyme, Fluorescent protein or Fluorescent Dye, with a hands-on time of under 30 seconds.

Principle of the Procedure:

Step 1: Prepare the Tissue Culture Supernatant. **Step 2:** Transfer the Protein A to the prepared supernatant and mix for 2 hours. **Step 3:** Transfer the solution into the column. **Step 4:** Wash the Protein A Resin. **Step 5:** Elute and Neutralize purified antibody. **Step 6:** Confirm antibody is in eluate using a test for protein. **Step 7:** Concentrate the antibody (Optional).

Protocols:

1. Amount of antibody that can be purified
The antibody to be purified should be in 10 to 50 ml of tissue culture supernatant. Up to 5 mg of antibody can be purified in each run.

2. Preparing the Tissue Culture Supernatant
Add the 10x Binding Buffer **AC049-001-B** to the Tissue Culture Supernatant (TCS). The volume to add is 1/10 of the volume of Tissue Culture Supernatant. For example, for 50 ml

of TCS add 5 ml of 10x Binding Buffer **AC049-001-B**. Mix by inversion.

3. Incubation of Sample with Resin
Add the AbSelect Resin **AC049-001-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 AbSelect 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 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 Wash Buffer **AC049-001-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 antibody is eluted in 1 ml fractions.
Place a collecting tube under the column and add 1 ml of Elution Buffer **AC049-001-E** (See Section 8).
Remove the collection tube and add 0.25 ml of Neutralization Buffer **AC049-001-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 **AC049-001-N** must be added as soon as possible to avoid prolonged exposure to low pH which can result in denaturation of the IgG. The protein normally elutes in tubes 1 and 2 but you should confirm this using a Test for Protein (See Section 8) before pooling any of the tubes.

7. Antibody Concentration (Optional)
If the concentration of the recovered antibody is low then it can very quickly and easily be concentrated using the antibody concentrator.

Step 1: Add 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 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.
NB. It is advisable not spin the 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.

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 **AC049-001-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 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.

Pictures:

Appendix 1: Protein A Affinity for immunoglobulins

Species	Ig	Binding strength
Rabbit	IgG	High
Human	IgG	High
Pig	IgG	High
Mouse	IgG_1	Medium/High
Mouse	IgG_{2a}	High
Mouse	IgG_{2b}	High
Mouse	IgG_3	Medium
Goat	IgG	Low
Sheep	IgG	Low
Rat	IgG	Low