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AbSelect Serum Antibody Purification System (3 Purifications, each up to 20mg IgG)

Catalog No.: AC050-003

Quantity: 1 set

Reaction: 3 Purifications, each up to 20mg IgG

Kit Contents:

AC050-003-R:	AbSelect Serum Protein A Resin
AC050-003-P:	1 Purification Column
AC050-003-B:	1 Bottle of 10x Binding Buffer
AC050-003-W:	1 Bottle of Wash Buffer
AC050-003-E:	1 Bottle of Elution Buffer
AC050-003-N:	1 vial of Neutralization Buffer

Background:

Antibodies generated from Ascites fluid and Serum are often supplied as crude formulations. Both Ascites fluid and Serum contain many substances that interfere with antibody labeling reactions. Successful labeling therefore requires antibodies to be purified prior to labeling. The AbSelect Serum system **AC050** is a fast and simple method to purify antibodies from these types of media.

AbSelect Serum is prepared by coupling highly purified Protein A to Agarose beads which can be used to purify IgG fractions from Ascites fluid and Serum. The method involves the capture of the antibody on the AbSelect Serum 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 Serum purification system 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 Serum or Ascites fluid. **Step 2:** Transfer the Protein A Resin to the prepared sample 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
Up to 20 mg of antibody can be purified in each run. The volume of sample required will depend on the host species (See Appendix 2).

2. Preparing the Serum or Ascites Fluid
Add the 10x Binding Buffer **AC050-003-B** to the Serum or Ascites fluid. The volume to add is 1/10 of the volume of the sample. For example, for 5 ml of Serum add 0.5 ml of 10x Binding

Buffer **AC050-003-B**. Mix by inversion. Note: For sample volumes of less than 5 ml, dilute the sample with Wash Buffer **AC050-003-W** to 5 ml before adding the 10x Binding Buffer **AC050-003-B**.

3. Incubation of Sample with Resin

Add the AbSelect Resin **AC050-003-R** to the Serum 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 Sample-Resin mix into the column. Sample volumes of more than 10 ml will have to be added in aliquots. The AbSelect Serum 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 **AC050-003-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 **AC050-003-E** (See Section 8).
Remove the collection tube and add 0.25 ml of Neutralization Buffer **AC050-003-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 **AC050-003-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 (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 our Antibody Concentration and Clean Up Kit (Cat.-N **AC048** available separately).

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 **AC050-003-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 2: Total IgG levels in normal Serum and Ascites fluid.

Species	Normal range IgG (mg/ml)	Suggested suitable volumes for product (ml)
Rabbit	12 to 15	1.3 to 1.7
Human	7 to 23	0.9 to 2.9
Mouse	2 to 5	4 to 10
Goat	18 to 24	0.8 to 1.1
Sheep	18 to 24	0.8 to 1.1
Rat	5 to 7	2.9 to 4
Ascites fluid	0.5 to 5	4 to 40

Appendix 1: Protein A Affinity for immunoglobulins.

Species	Ig	Binding strength
Rabbit	IgG	High
Human	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