Overview of Procedure

For Montage® Antibody Purification Kit and Spin Columns with PROSEP®-A Media

Materials Supplied with both Kit and Spin Columns

- PROSEP-A media plugs (immobilized recombinant Protein A)
- Insertion tool
- Spin columns
- Centrifuge tubes

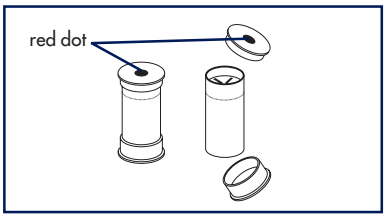
Materials Supplied only in the Kit

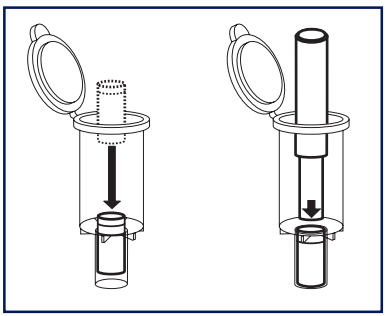
- Steriflip®-GP filters, 0.22 µm (Millipore cat. no. SCGP 005 25)
- Amicon® Ultra-15 centrifugal filter device with 30,000 NMWL (Cat. no. UFC9 030 24)
- Binding buffer A: 1.5 M Glycine/NaOH, 3 M NaCl, pH 9.0
- Elution buffer B1: 0.1 M Sodium citrate pH 5.5
- Elution buffer B2: 0.2 M Glycine/HCl pH 2.5
- Neutralization buffer C: 1 M Tris/HCl pH 9.0

Additional Materials Required

- Vacuum pressure pump or uniform vacuum source (Cat. no. WP61 115 60 or equivalent)
- Centrifuge with swinging bucket rotor capable of handling 50 mL tubes
- Volumetric pipettes
- 15 mL screw-cap centrifuge tube

Loading the Media Plug into the Spin Column





1. Unwrap the sealing film from both ends of the plug.
2. Remove top and bottom caps.
   NOTE: Once the caps are removed, the top end can be identified because it is recessed (approximately 2 mm deep).
3. Insert the plug into the spin column with the top (recessed) end uppermost.
4. Push the plug fully into the tapered end of the spin column using the insertion tool.

To remove the plug from the spin column, insert the tool into the bottom of the spin column and push upward.

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Antibody Purification Protocol

After loading the plug into the spin column and placing the spin column into a centrifuge tube, follow the procedure below.

PRE-EQUILIBRATION

1. Equilibrate the PROSEP-A media with 10 mL Binding Buffer A by centrifuging the spin column at 500 × g for 5 minutes.

   Note: If using one spin column, ensure that the spin column is counterbalanced with a unit of equal weight (adjusted with distilled water) and without a plug.

CLARIFICATION OF SAMPLE

2. Pre-filter the sample (e.g., tissue culture supernatant, serum, or ascites) through a 0.22 µm Steriflip-GP filter device to remove any debris immediately before loading the sample.

SAMPLE LOADING

3. Dilute the filtered sample 1:1 v/v in Binding Buffer A. (For example, add 10 mL filtered sample to 10 mL buffer.) Pipette the sample into the spin column. Centrifuge the spin column at 100–150 × g for 20 minutes.

   Notes: It may be necessary to increase the spin time or spin speed if any sample remains above the plug. If using one spin column, ensure that the spin column is counterbalanced with a unit of equal weight (adjusted with distilled water) and without a plug.

WASHING

4. Wash the spin column by centrifuging the spin column for 5 minutes at 500 × g with 20 mL Binding Buffer A to remove unbound contaminants.

   Note: For more thorough washing, centrifuge spin column two times with 10 mL Binding Buffer A for 2 minutes at 500 × g, instead of spinning 20 mL once.

ELUTION

- To purify mouse IgG1, rat IgG1, rat IgG2a, rat IgG2b and bovine IgG1, use both elution steps 5 & 6.
- To purify mouse IgG2a, mouse IgG2b, mouse IgG3, rat IgG2c, human IgG1-IgG4, rabbit IgG, guinea pig IgG1, guinea pig IgG2, bovine IgG2 and any other IgGs, use elution step 6 only.

5. Elute the bound IgG with 10 mL Elution Buffer B1 directly into a fresh centrifuge tube containing 0.5 mL Neutralization Buffer C to bring the sample to neutral pH. Centrifuge the spin column for 5 minutes at 500 × g.
6. Elute the bound IgG with 10 mL Elution Buffer B2 directly into a fresh centrifuge tube containing 1.3 mL Neutralization Buffer C to bring the sample to neutral pH. Centrifuge the spin column for 5 minutes at 500 × g.

REGENERATION

7. Wash the media with 10 mL Elution Buffer B2 by centrifuging the spin column at 500 × g for 5 minutes. Re-equilibrate the media with 5 mL of Binding Buffer A by centrifuging the spin column at 500 × g for 2 minutes. Return to step 1 for immediate re-use or for later use, store the plugs without their end caps in 5 mL of Binding Buffer A in a 15 mL screw-capped tube.

DESALTING AND CONCENTRATING

8. If necessary, de-salt and concentrate the antibody preparation using the Amicon Ultra-15 centrifugal filter device with 30,000 NMWL.

See the Millipore web site at www.millipore.com/montagePROSEP for user guides containing complete information about using the following Millipore products:

Product	Publication Number
Montage Antibody Purification Kit with PROSEP-A Media	P36486
Steriflip Filter Unit	P35824
Amicon Ultra-15 Centrifugal Filter Device	P36375

MILLIPORE