Easy-to-read Midi Purification Protocol E.g. Human serum

Fraction	*Volume*	*Step*	*RCF*	*Spin Time*
Pre-equilibration	10 ml	BBA pH 9.0	500 g	3 min
Sample Loading	20 ml	1:1 serum: BBA pH 9.0	100 g	30 min
Wash #1	10 ml	BBA pH 9.0	500 g	3 min
Wash #2	10 ml	BBA pH 9.0	500 g	3 min
Final Eluate #1	10 ml	EB2 → 1.3 ml NBC	500 g	3 min
Final Eluate #2	10 ml	EB2 → 1.3 ml NBC	500 g	3 min

Easy-to-read Midi Regeneration Protocol

Fraction	*Volume*	*Step*	*RCF*	*Spin Time*
Clean-up	10 ml	EB2 pH 2.5	500 g	3 min
Wash	10 ml	BBA pH 9.0	500 g	3 min

pro chem
ADVANCED PROTEIN SEPARATIONS

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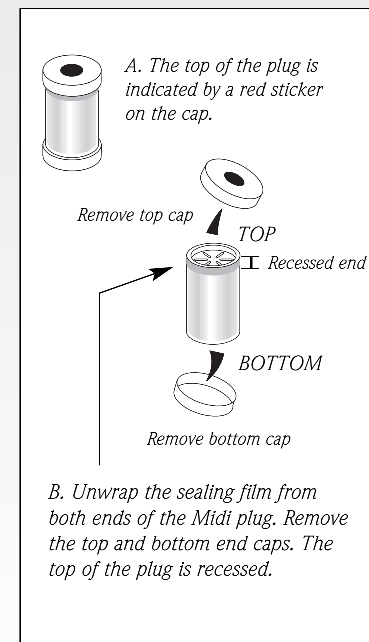
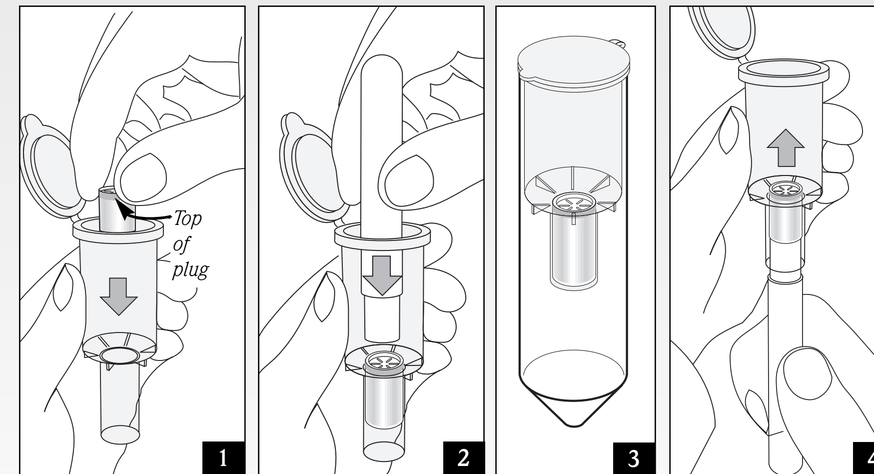
MIDI

Loading and removing the plug from the spin column



B. Unwrap the sealing film from both ends of the Midi plug. Remove the top and bottom end caps. The top of the plug is recessed.



Place the plug into the spin column with the recessed end uppermost. Push the plug **FULLY** *into the tapered end of the spin column using the plug insertion tool. It is now ready for pre-equilibration with binding buffer followed by centrifugation. After use, the plug is removed using the plug insertion tool.*

PROTEIN A

Step by step protocol for Midi Spin Columns

RESIN PLUG LOADING
1. Load the pre-packed resin Midi plug containing immobilized recombinant Protein A resin into the barrel of the Proteus spin column using the insertion tool.

PRE-EQUILIBRATION (Total spin time = 3 mins)
2. Equilibrate the Protein A spin column with 10 ml binding buffer A, pH 9.0 by centrifuging the spin column at 500 g for 3 min*.

CLARIFICATION OF SAMPLE
3. Filter 12-15 ml sample through a single 1.2 μm (25 mm diameter) syringe filter to remove any cellular debris. Then, filter the partially clarified sample through a single 0.2 μm (25 mm diameter) syringe filter.

   N.B: Protein precipitation is common during storage and repeated freeze/thaw cycles in ascites, sera and tissue culture supernatants. As with all forms of chromatography, it is important that the sample is filtered through a final 0.2 μm syringe filter **immediately** before loading it on to the spin column.

* If 1 spin column is to be used, ensure that the spin column is counterbalanced with a unit of equal weight (adjusted with distilled water) and without a Protein A resin plug.

SAMPLE LOADING (Total spin time = 30 mins)
4. Dilute the sample 1:1 (v/v; eg. add 10 ml 0.2 μm filtered sample to 10 ml binding buffer A, pH 9.0). Mix by inverting the capped tube 3-4 times. Pipette the 20 ml sample into the spin column. Centrifuge the spin column at 100 g for 30 min.

   N.B: Increase the spin time or speed if any sample remains above the plug.

WASHING (Total spin time = 6 mins)
5. Wash the spin column twice with 10 ml binding buffer A, pH 9.0 to remove unbound contaminants by centrifuging the Proteus spin columns for 3 min at 500 g. The unbound wash will contain non-immunoglobulin components.

ELUTION (Total spin time = 6 mins)
For purifying mouse IgG1, rat IgG1, rat IgG2a, rat IgG2b and bovine IgG1, use both elution steps 6 and 7.

ELUTION (Total spin time = 6 mins)
For purifying unassigned IgG, 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, proceed to elution step 7 only.

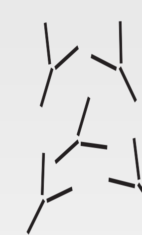
6. Elute the bound IgG with 10 ml elution buffer B1 directly into a fresh centrifuge tube containing 0.5 ml neutralization buffer C to bring the pH of the sample to approximately 7.5. Centrifuge the Proteus spin column for 3 min at 500 g. Swirl the tube to ensure thorough mixing of the final eluate with neutralization buffer C. Repeat this elution step.

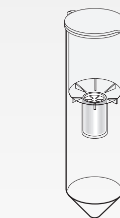
   N.B: Do not pool the two eluate fractions if you want to recover ***concentrated*** purified antibody.

7. 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 pH of the sample to approximately 7.5. Centrifuge the Proteus spin column for 3 min at 500 g. Swirl the tube to ensure thorough mixing of the final eluate with neutralization buffer C. Repeat this elution step.

   N.B: Do not pool the two eluate fractions if you want to recover ***concentrated*** purified antibody.

Pure Antibody





Used Spin Column

DESALTING AND CONCENTRATING THE PURIFIED ANTIBODY
8. If necessary, de-salt and concentrate the antibody preparation using the 30 kDa MWCO ultrafiltration spinner supplied. Add 0.05-0.2 % w/v sodium azide if the antibodies are to be stored at 2-8 °C.
   We recommend freezing the antibodies in small aliquots in 10-50 % glycerol at -20 °C for long term storage.

REGENERATION OF THE PROTEIN A MIDI PLUG
9. Wash the Midi plugs with 10 ml elution buffer B2 (pH 2.5) by centrifuging the spin columns at 500 g for 3 min. Then wash the plugs with 10 ml binding buffer A (pH 9.0) by centrifuging the spin columns at 500 g for 3 min. Proceed to the pre-equilibration step of another bind-wash-elute cycle if the plugs are to be re-used immediately. After regeneration, plugs can also be stored, without their end caps, in binding buffer A or in 0.1 % sodium azide (made up in distilled water) at 2-8 °C until further use.

Buffers

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

PROTEUS