

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

<i>Fraction</i>	<i>Volume</i>	<i>Step</i>	<i>RCF</i>	<i>Spin Time</i>
Pre-equilibration	10 ml	BBA pH 7.4	500 g	3 min
Sample Loading	20 ml	1:1 serum: BBA pH 7.4	150 g	30 min
Wash #1	10 ml	BBA pH 7.4	500 g	3 min
Wash #2	10 ml	BBA pH 7.4	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

<i>Fraction</i>	<i>Volume</i>	<i>Step</i>	<i>RCF</i>	<i>Spin Time</i>
Clean-up	10 ml	EB2 pH 2.5	500 g	3 min
Wash	10 ml	BBA pH 7.4	500 g	3 min

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ADVANCED PROTEIN SEPARATIONS

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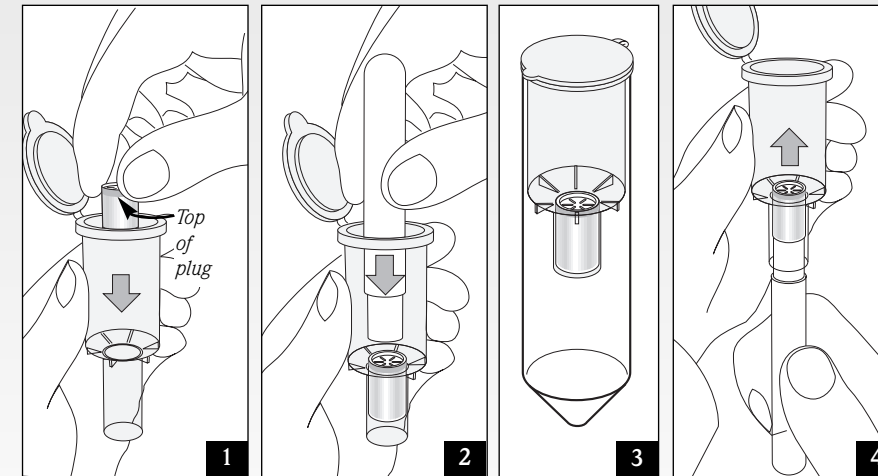
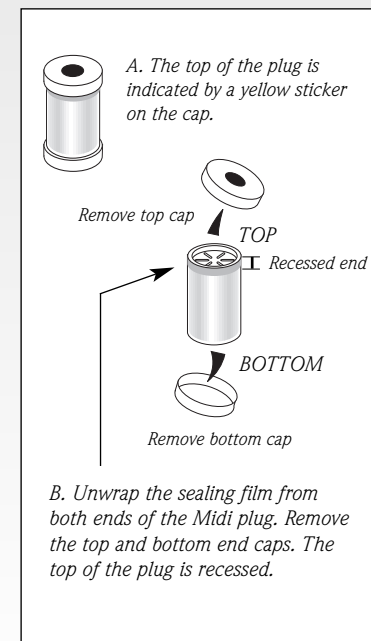
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MIDI

Loading and removing the plug from the spin column

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 G

Step by step protocol for Midi Spin Columns

RESIN PLUG LOADING

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

PRE-EQUILIBRATION (Total spin time = 3 mins)

2. Equilibrate the Protein G spin column with 10 ml binding buffer A, pH 7.4 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.

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 7.4). Mix by inverting the capped tube 3-4 times. Pipette the 20 ml sample into the spin column. Centrifuge the spin column at 150 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 7.4 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)

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 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.

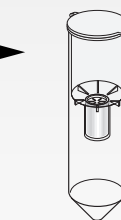
Buffers

Binding Buffer A: 0.1 M Sodium phosphate, 0.15 M NaCl, pH 7.4

Elution Buffer B2: 0.2 M Glycine/HCl pH 2.5

Neutralization Buffer C: 1 M Tris/HCl pH 9.0

Pure Antibody



Used Spin Column

DESALTING AND CONCENTRATING THE PURIFIED ANTIBODY

7. 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 G MIDI PLUG

8. 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 7.4) 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.

* 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 G resin plug.

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