

Easy-to-read Mini Purification Protocol E.g. Human serum				
Fraction	Volume	Step	RCF	Spin Time
Pre-equilibration #1	0.65 ml	BBA pH 9.0	1,800 g	1 min
Pre-equilibration #2	0.65 ml	BBA pH 9.0	1,800 g	1 min
Sample Loading	0.65 ml	1:1 serum: BBA pH 9.0	640 g	6 min
Wash #1	0.65 ml	BBA pH 9.0	1,800 g	2 min
Wash #2	0.65 ml	BBA pH 9.0	1,800 g	2 min
Wash #3	0.65 ml	BBA pH 9.0	1,800 g	2 min
Final Eluate #1	0.5 ml	EB2 → 65 µl NBC	1,800 g	2 min
Final Eluate #2	0.5 ml	EB2 → 65 µl NBC	1,800 g	2 min

Easy-to-read Mini Regeneration Protocol				
Fraction	Volume	Step	RCF	Spin Time
Clean-up #1	0.65 ml	EB2 pH 2.5	1,800 g	2 min
Clean-up #2	0.65 ml	EB2 pH 2.5	1,800 g	2 min
Wash #1	0.65 ml	BBA pH 9.0	1,800 g	2 min
Wash #2	0.65 ml	BBA pH 9.0	1,800 g	2 min

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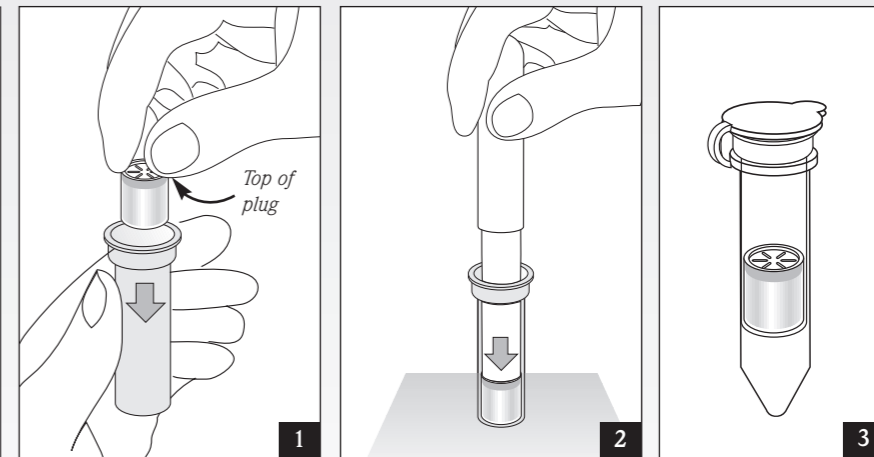
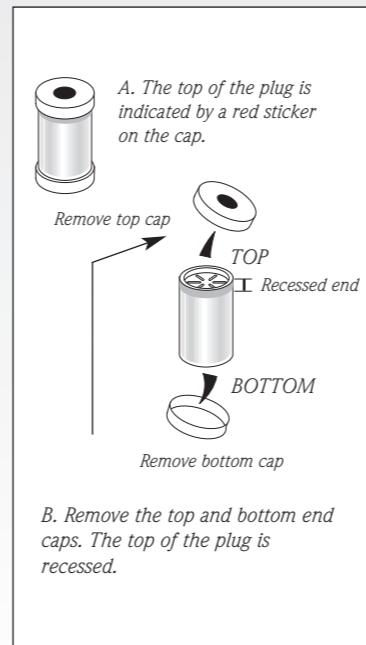
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MINI

Loading the plug into 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.

PROTEIN A

Step by step protocol for Mini Spin Columns

RESIN PLUG LOADING

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

PRE-EQUILIBRATION (Total spin time = 2 mins)

2. Equilibrate the Protein A spin column with 0.65 ml binding buffer A, pH 9.0 by centrifuging the spin column at 1,800 g (4,400 rpm in a Heraeus Biofuge Pico or 5,000 rpm in a Sanyo MSE Micro Centaur) for 1 min*. Repeat this pre-equilibration step with 0.65 ml binding buffer A, pH 9.0 at 1,800 g for 1 min.

CLARIFICATION OF SAMPLE

3. Filter 1 ml sample through a single 0.2 μ m syringe filter to remove any cellular debris.

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 μ m syringe filter **immediately** before loading it on to the spin column.

SAMPLE LOADING (Total spin time = 6 mins)

4. Dilute the sample 1:1 (v/v; eg. add 0.5 ml 0.2 μ m filtered sample to 0.5 ml binding buffer A, pH 9.0). Mix by inverting the capped tube 3-4 times. Pipette the 0.65 ml sample into the spin column. Centrifuge the spin column at 640 g (2,600 rpm in a Heraeus Biofuge Pico or 3,000 rpm in a Sanyo MSE Micro Centaur) for 6 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 three times with 0.65 ml binding buffer A, pH 9.0 to remove unbound contaminants by centrifuging the Proteus spin columns for 2 min at 1,800 g (4,400 rpm in a Heraeus Biofuge Pico or 5,000 rpm in a Sanyo MSE Micro Centaur). The unbound wash will contain non-immunoglobulin components.

ELUTION (Total spin time = 4 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 = 4 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.

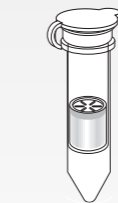
6. Elute the bound IgG with 0.5 ml elution buffer B1 directly into a fresh centrifuge tube containing 25 μ l neutralization buffer C to bring the pH of the sample to approximately 7.5. Centrifuge the Proteus spin column for 2 min at 1,800 g. Swirl the tube to ensure thorough mixing of the final eluate 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 0.5 ml elution buffer B2 directly into a fresh centrifuge tube containing 65 μ l neutralization buffer C to bring the pH of the sample to approximately 7.5. Centrifuge the Proteus spin column for 2 min at 1,800 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 MINI PLUG

9. Wash the Mini plugs twice with 0.65 ml elution buffer B2 (pH 2.5) by centrifuging the spin columns at 1,800 g for 2 min. Then wash the plugs twice with 0.65 ml binding buffer A (pH 9.0) by centrifuging the spin columns at 1,800 g for 2 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

* If 1 spin column is to be used, ensure that the spin column is counterbalanced in the microfuge with a microcentrifuge tube filled with the correct level of water.