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

prochem
ADVANCED PROTEIN SEPARATIONS

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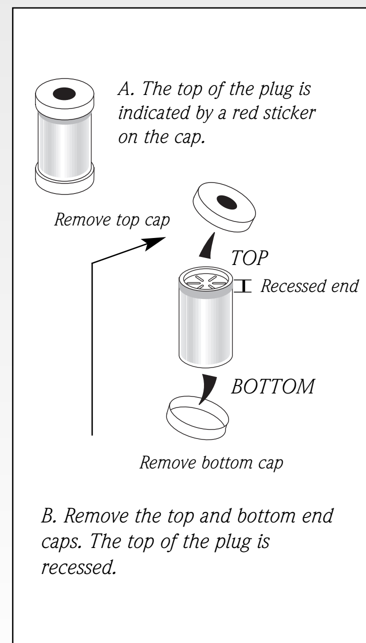
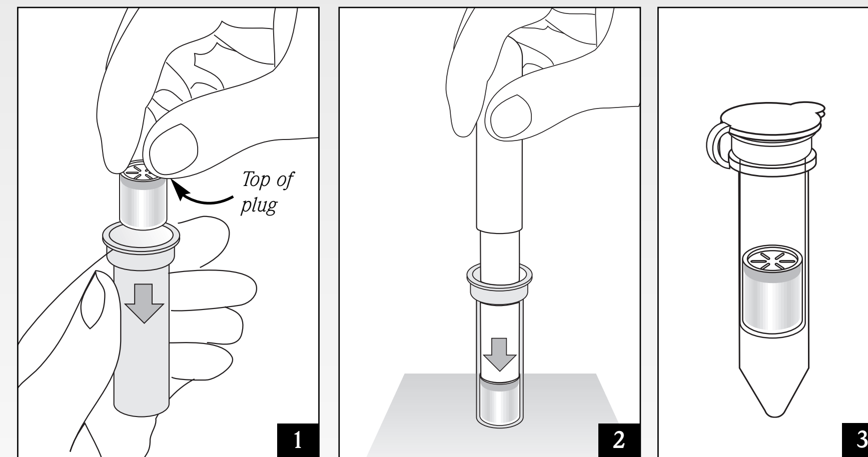
MINI

Loading the plug into the spin column



B. 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.*

PROTEINA

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 μm 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 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 in the microfuge with a microcentrifuge tube filled with the correct level of water.

SAMPLE LOADING (Total spin time = 6 mins)

4. Dilute the sample 1:1 (v/v; eg. add 0.5 ml 0.2 μm 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.

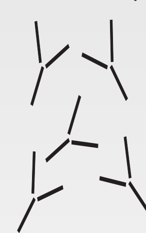
6. Elute the bound IgG with 0.5 ml elution buffer B1 directly into a fresh centrifuge tube containing 25 μl neutralization buffer C to bring the pH of the sample to approximately 7.5. Centrifuge the Proteus spin column for 2 min at 1,800 g. Swirl the tube to ensure thorough mixing of the final eluate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

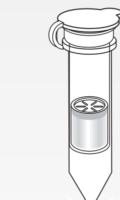
   N.B: Do not pool the two eluate fractions if you want to recover ***concentrated*** purified antibody.

Pure Antibody



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.



Used Spin Column

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

PROTEUS