

The following suggested protocol has been optimized using maximum and minimum protein concentrations of 2.5 mg/mL and 0.01 mg/mL respectively and is provided to demonstrate the potential uses of the ProTrap XG.

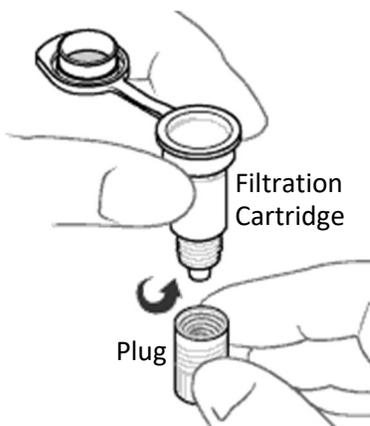
More dilute protein solutions require extra care, please contact Proteoform Scientific at [support@proteoform.com](mailto:support@proteoform.com) or +1 902 442 4664 for a customized protocol. Additional protocols and the ProTrap XG User Manual are available at [proteoform.com](http://proteoform.com)

### PREPARATION NOTES

- Spin speeds are based on a standard benchtop microcentrifuge with 24 x 1.5/2.0 mL rotor.
- Times provided are guidelines only.
- If more than a few microliters of liquid remains in the Filtration Cartridge after any spin, return it to the centrifuge and repeat the spin, or consider increasing the spin speed. 3000  $\times g$  (6000 rpm) is recommended for subsequent spins and the ProTrap XG has been tested up to 9000  $\times g$  (10,000 rpm).
- The capacity of the ProTrap XG is 250  $\mu g$  of protein.

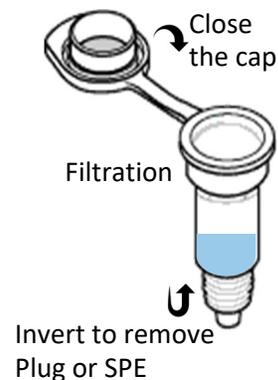
### ASSEMBLING THE PROTRAP XG

The ProTrap's interchangeable components are packaged separately. Below is some guidance on assembling and using the components together in workflows.

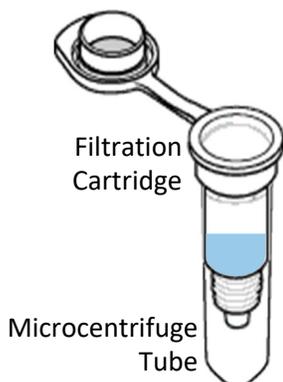


The Plug screws onto the base of the Filtration Cartridge. To ensure a tight seal, give a firm twist by hand.

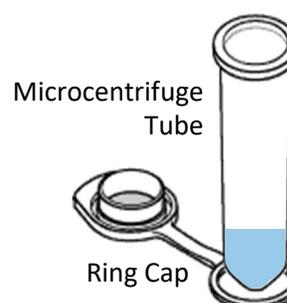
The SPE Cartridge is attached and removed in the same way



After sample and reagents have been added to the Filtration Cartridge, cap it and invert before unscrewing the Plug or SPE Cartridge.



Place the Filtration Cartridge into a Microcentrifuge Tube prior to loading into the centrifuge.



A Ring Cap is provided to conveniently store your sample in the Microcentrifuge Tube. Slide the Ring Cap on to the Microcentrifuge Tube from the bottom.

## MATERIALS REQUIRED

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*All chemicals and reagents should be ACS grade/HPLC grade or better.*

Acetone

5 M NaCl in water

## PROTEIN PRECIPITATION IN ACETONE

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To your protein solution, before adding it to the Filtration Cartridge, add sufficient 5 M NaCl to bring the NaCl concentration to 50 mM before adding your sample to the Filtration Cartridge.

Screw a Plug onto the base of the Filtration Cartridge.

Transfer 100  $\mu$ L of the salted protein to the plugged Filtration Cartridge.

Add 400  $\mu$ L room temperature acetone.

Cap the Filtration Cartridge and rock gently, tilting no more than 45°, to combine the solvents.

Insert the Filtration Cartridge in the Microcentrifuge Tube, allow 30 minutes for the protein to fully aggregate at room temperature.

With Plug attached, centrifuge at 2500  $\times$ g (5000 rpm)  $\times$ 2 minutes.

Remove Filtration Cartridge from the Microcentrifuge Tube, invert, and unscrew the Plug.

Return the capped Filtration Cartridge to the Microcentrifuge Tube and centrifuge at 500  $\times$ g (2000 rpm)  $\times$ 5 minutes. Discard the flow through solvent. If any solvent remains in the Filtration Cartridge, re-spin the unit for 2-5 minutes at 3000  $\times$ g (6000 rpm).

Wash the protein pellet with 400  $\mu$ L acetone. Immediately centrifuge at 500  $\times$ g (2000 rpm)  $\times$ 2 minutes. Discard the flow through wash solvent.

Intact proteins may be resolubilized using the **Resolubilization of Intact Protein Protocol**, or subject to enzymatic digestion within the **Protein Digestion Protocol**.

**Do not allow the membrane in the Filtration Cartridge to dry out between steps. Proceed immediately to your next protocol.**



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