ProTrap XG

Resolubilization of Intact Protein

PROTOCOL

The following suggested protocol has been optimized using maximum and minimum protein concentrations of 0.5 mg/mL and 0.01 mg/mL respectively and is provided to demonstrate the potential uses of the ProTrap XG.

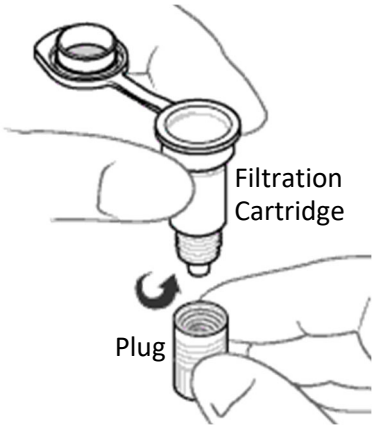
More dilute protein solutions require extra care, please contact Proteoform Scientific at support@proteoform.com or +1 902 442 4664 for a customized protocol. Additional protocols and the ProTrap XG User Manual are available at proteoform.com

PREPARATION NOTES

- The ProTrap XG device is optimized to process 50 µg of protein.
- 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 ×g (6000 rpm) is recommended for subsequent spins and the ProTrap XG has been tested up to 9000 ×g (10,000 rpm).

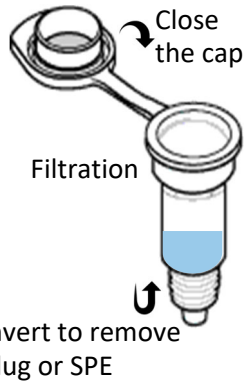
ASSEMBLING THE PROTRAP XG

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

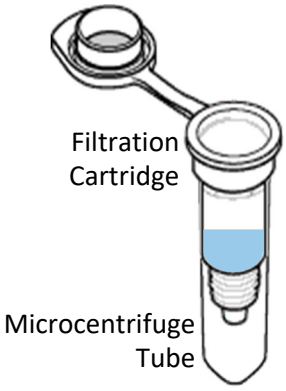


The Plug screws onto the base of the Filtration Cartridge. To ensure a tight seal, give a firm twist by hand.

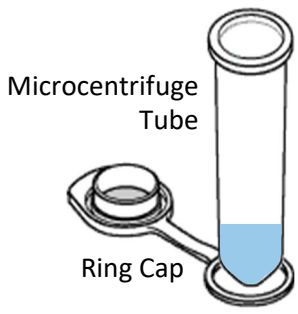
The SPE Cartridge is attached and removed in the same way



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

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

80% Formic acid in water (chilled to –20°C)

Chilled water

RESOLUBILIZATION OF INTACT PROTEIN

This procedure applies to samples following solvent precipitation in the **Protein Precipitation in Acetone Protocol** provided.

Once the sample has been precipitated using the provided protocol, with the Plug still attached to the base of the Filtration Cartridge, add 50 µL of cold (-20°C) 80% formic acid in water.

Cap the Filtration Cartridge, place in freezer for 10 minutes, then sonicate for 1 minute.

Add 450 µL chilled water; cap and vortex to mix the solvent.

Intact proteins may be directly recovered in a clean Microcentrifuge Tube, centrifuging at 350 ×g (2000 rpm) ×5 minutes.

Resolubilized proteins may also be subject to SPE using the provided SPE Cartridge and the **SPE Protein/Peptide Clean-Up Protocol**.

REFERENCES

Doucette AA, Vieira DB, Orton DJ, Wall MJ "Resolubilization of Precipitated Intact Membrane Proteins with Cold Formic Acid for Analysis by Mass Spectrometry" *Journal of Proteome Research* 201, 13, 6001-3012

Donnelly et al "Best practices and Benchmarks for intact protein analysis for top-down mass spectrometry" *Nature Methods* 2019 16: 587

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