ProTrap XG

Protein Digestion PROTOCOL

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.

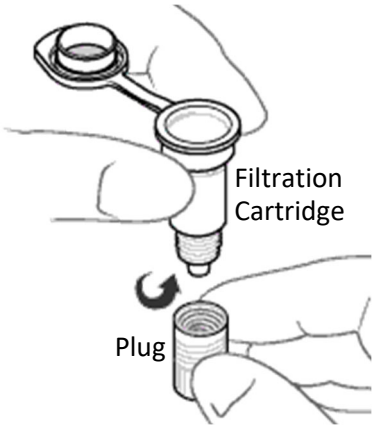
More dilute protein solutions require extra care, please contact Proteoform Scientific at support@proteoform.com or +1 902 442 4664 for a customized protocol. Additional protocols and the ProTrap XG User Manual are available at proteoform.com

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.
- It is essential that once primed the SPE cartridge is not spun to complete dryness.
- 3000 ×g (6000 rpm) is recommended for subsequent spins and the ProTrap XG has been tested up to 9000 ×g (10,000 rpm).
- The capacity of the ProTrap XG is 250 µg of protein.

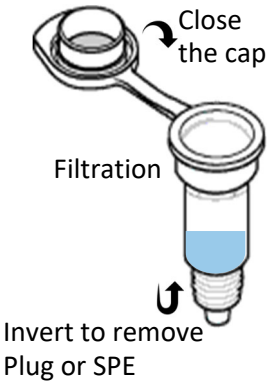
ASSEMBLING THE PROTRAP XG

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

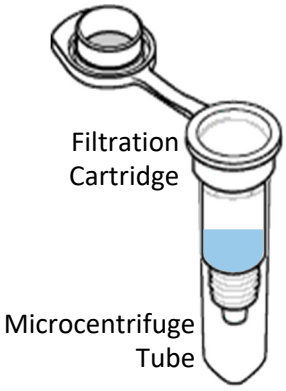


The Plug screws onto the base of the Filtration Cartridge. To ensure a tight seal, give a firm twist by hand.

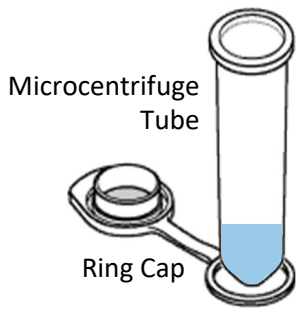
The SPE Cartridge is attached and removed in the same way



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

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

8 M urea in 50 mM Tris-HCl pH 8.0 with 20 mM DTT freshly prepared

500 mM Iodoacetamide in 50 mM Tris-HCl pH 8.0 freshly prepared

50 mM Tris-HCl pH 8.0 with 2 mM $CaCl_2$

Trypsin diluted in 50 mM Tris HCl pH 8.0 with 2 mM $CaCl_2$

10% TFA

PROTEIN DIGESTION

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

Once the sample has been precipitated using the provided protocol, with the Plug still attached to the Filtration Cartridge:

Solubilize and Reduce your sample: Add 50 µL 8 M urea in 50 mM Tris-HCl pH 8.0 with 20 mM DTT. Vortex for 30 seconds and sonicate for 10 minutes. Incubate at 37°C for 1 hour to reduce the disulphide bonds.

Alkylate: Add 10 µL 500 mM iodoacetamide in 50 mM Tris-HCl pH8.0. Incubate for 15 minutes in the dark at room temperature.

Dilute: Add 400 µL 50 mM Tris-HCl pH 8.0, 2 mM $CaCl_2$

Digest: Add Trypsin diluted in 50 mM Tris HCl pH 8.0 2 mM $CaCl_2$ at a ratio of 100:1 protein: enzyme. Incubate in a water bath overnight (16-18 hours).

Stop and Acidify: Add 4.7 µL of 10% TFA. Ensure pH < 3.5.

Recover or SPE: Peptides may be recovered by centrifuging after removing Plug and placing the Filtration Cartridge in the provided Microcentrifuge Tube (2500 ×g (5000 rpm) ×5 minutes), **OR** subject to SPE cleanup using the **SPE Protein/Peptide Clean-Up Protocol** provided.

Note: If your optimized digestion protocol differs in time, temperature, reducing or alkylating reagent, or concentration of Trypsin, get in touch with our team at support@proteoform.com to confirm that your process can be transferred to the ProTrap XG with no issues.

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