ProTrap XG

Bottom Up Sample Prep
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.

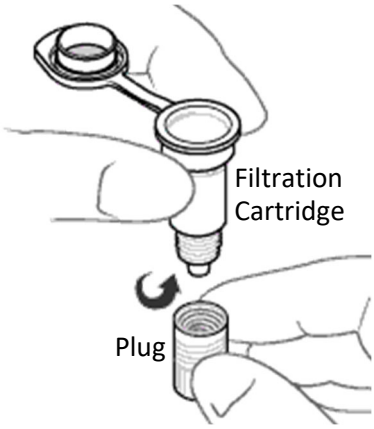
Our team works with customers to optimize protocols for your workflow, sample type, and research goals. Connect with us at sales@allumiqs.com to learn more about how we can help.

PREPARATION NOTES

- The ProTrap XG device is optimized to process 50 µg of protein.
- For reproducible and maximal protein precipitation, the maximum SDS content in your sample during precipitation should be 1%. If your extraction or lysis buffer contains more than 1% SDS, dilute it with a buffer containing a maximum ionic strength of 300 mM.
- 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).

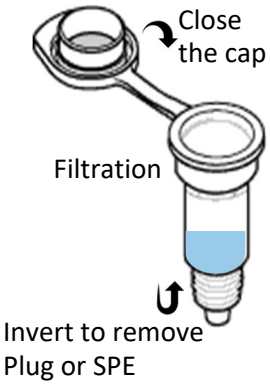
ASSEMBLING THE PROTRAP XG

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

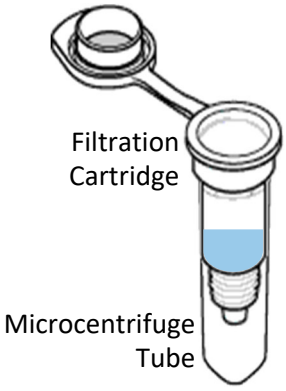


The Plug screws onto the base of the Filtration Cartridge. To ensure a tight seal, give a firm twist by hand.

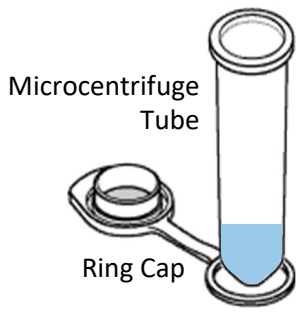
The SPE Cartridge is attached and removed in the same way



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.*

Acetone
5 M NaCl in water
8 M urea in 50 mM Tris-HCl pH 8.0 with 10 mM DTT freshly prepared
90 mM Iodoacetamide in 50 mM Tris-HCl pH 8.0 freshly prepared
70 mM DTT in 50 mM Tris-HCl pH 8.0
50 mM Tris-HCl pH 8.0
Trypsin diluted in 50 mM Tris HCl pH 8.0
Formic acid

BOTTOM UP SAMPLE PREP PROTOCOL

Prepare and Precipitate your Sample: For reproducible and maximal protein precipitation, the maximum SDS content in your sample during precipitation should be 1%. If your extraction or lysis buffer contains more than 1% SDS, dilute it with a buffer containing a maximum ionic strength of 300 mM. For the best experience, your sample should contain at least 50 mM NaCl. If you need to add NaCl, use a 5 M NaCl solution.

Do not allow the membrane in the Filtration Cartridge to dry out between steps.

Screw a Plug onto the base of the Filtration Cartridge.

Transfer 100 µL of the diluted sample protein to the plugged Filtration Cartridge.

Add 400 µL 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 ×g (5000 rpm) ×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 ×g (2000 rpm) ×3 minutes. Discard the flow through solvent. If any solvent remains in the Filtration Cartridge, re-spin the unit for 2-5 minutes at 3000 ×g (6000 rpm).

Wash the protein pellet with 400 µL acetone. Immediately centrifuge at 500 ×g (2000 rpm) ×2 minutes. Discard the flow through wash solvent.

Replace the plug.

Solubilize and Reduce your sample: Add 50 µL 8 M urea in 50 mM Tris-HCl pH 8.0 with 10 mM DTT. Gently pipette up and down, taking care not to cause foaming, then sonicate for 10 minutes. Incubate at 37°C for 1 hour to reduce the disulphide bonds.

Alkylate: Add 10 µL 90 mM iodoacetamide in 50 mM Tris-HCl pH8.0 (15 mM final concentration). Incubate for 15 minutes in the dark at room temperature. Quench the reaction by adding 10 µL 70 mM DTT (10 mM final concentration).

Dilute: Add 430 µL 50 mM Tris-HCl pH 8.0.

Digest: Add Trypsin diluted in 50 mM Tris HCl pH 8.0 at a ratio of 30:1 protein: enzyme. Incubate in a 37°C water bath overnight (16-18 hours).

Stop and Acidify: Add 10 µL formic acid. 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 OPTIONAL ProTrap XG SPE Cartridge and 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@allumiqs.com to confirm that your process can be transferred to the ProTrap XG with no issues.*

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