PROTEOFORM SCIENTIFIC

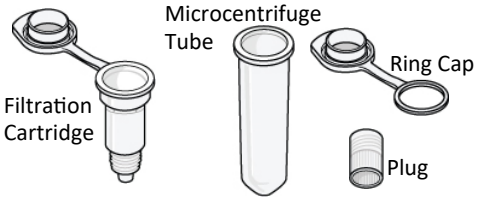
ProTrap XG USER GUIDE

The ProTrap XG is a disposable filtration/extraction cartridge, which operates within a benchtop microcentrifuge.
For Research Use Only.

CONTENTS

The ProTrap XG is available in three pack sizes and each kit contains the following components:

PXG-0001 ProTrap XG 10PK
PXG-0002 ProTrap XG 50PK
PXG-0003 ProTrap XG 100PK



SPE Accessory:

OPTIONAL
(included in some trial kits)
Priming Cartridge + SPE Cartridge

REAGENTS REQUIRED FOR SAMPLE PREPARATION

BOTTOM-UP WORKFLOWS

Precipitation: sodium chloride, acetone

Digestion: 8M Urea, 100 m M Tris pH 8.0, Reducing agent (e.g. 200 mM DTT or TCEP or alternate), alkylating reagent (e.g. 200 mM iodoacetamide or alternate), Trypsin, 10% trifluoroacetic acid (TFA)

Optional SPE: acetonitrile, 0.1 % TFA, 5% acetonitrile/0.1% TFA, 50% acetonitrile/0.1% TFA

TOP-DOWN WORKFLOWS

Precipitation: sodium chloride, acetone

Resolubilization: 80% formic acid

Optional SPE: acetonitrile, 0.1% TFA, 5% acetonitrile/0.1% TFA, 30% isopropanol/42% formic acid, 40% isopropanol/36% formic acid

STANDARD LAB EQUIPMENT REQUIRED FOR SAMPLE PREPARATION

Centrifuge, benchtop, capable of 350 to 9000 ×g (2000 to 10,000 rpm)

Pipettes and appropriate tips

1.7 or 2 mL Microcentrifuge tubes for waste collection

Waterbath or heat block at 37°C for Trypsin digestion, if appropriate

Ultrasonic Bath

Freezer

Vortex mixer

Personal protective equipment

Biohazard waste container

Organic waste container

Disinfectant

PREPARATION AND ASSEMBLY NOTES

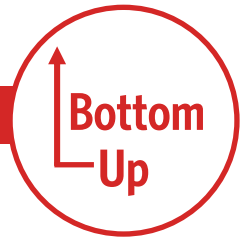
The attachments, the Plug and the optional SPE Cartridge, screw onto the base of the Filtration Cartridge. To ensure a tight seal, once the attachments are connected give a firm twist by hand.

When solvent is present in the Filtration Cartridge, flip the cartridge upside down with the cap on, prior to unscrewing the attachments.

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

SUGGESTED PROTOCOLS

- **The following suggested protocols have been optimized using 100 µL of a maximum and minimum protein concentrations of 2 mg/mL and 0.01 mg/mL respectively and are provided to demonstrate the potential use of the ProTrap XG .**
- **More dilute protein solutions require extra care, please contact us (info@proteoform.com) for a special protocol.**
- Spin speeds are based on a standard fixed-angle 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 optional 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 200 µg of protein.



Protein Precipitation in Acetone Protocol

Reagents required in this protocol: sodium chloride, acetone

- Screw a Plug onto the base of the Filtration Cartridge.
- If your sample contains no sodium chloride, add NaCl to give a final concentration of 20 to 100 mM.
- Transfer 100 µL of the salted protein to the plugged Filtration Cartridge.
- Add 400 µL room temperature acetone.
- Cap the Filtration Cartridge and rock gently 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 350 ×g (2000 rpm) ×5 minutes. Discard the flow through solvent.
- Wash the protein pellet with 400 µL acetone. Immediately centrifuge at 350 ×g (2000 rpm) ×2 minutes. Discard the flow through wash solvent.

Proceed immediately to the **Protein Digestion Protocol**.

Protein Digestion Protocol

This procedure applies to samples following solvent precipitation

Reagents required in this protocol: 8M Urea, 100 m M Tris pH 8.0, Reducing agent (e.g. 200 mM DTT or TCEP or alternate), alkylating reagent (e.g. 200 mM iodoacetamide or alternate), Trypsin, 10% trifluoroacetic acid (TFA)

- With Plug attached, add 100 µL 8 M urea to a precipitated sample.
- Cap the Filtration Cartridge, vortex 30 seconds, and then sonicate an additional 10 minutes. Let sit at room temperature for an additional 30 minutes.
- Add 400 µL of 100 mM Tris buffer (pH 8), cap and vortex briefly to mix.
- Optional: Reduce/alkylate disulfide bonds as per standard protocols (e.g. dithiothreitol (DTT)/iodoacetamide).
- Add tryspin at 50:1 (protein: enzyme by mass). Cap the device, incubate in a warm water bath as per conventional solution digestion (e.g. 37°C, 1 hour to overnight).
- Stop the reaction by acidifying with trifluoroacetic acid (TFA) (final 1%).
- Peptides may be recovered by centrifuging (Plug removed, 2500 ×g (5000 rpm) ×5 minutes), or subject to OPTIONAL SPE cleanup with the SPE Cartridge accessory (available in some trial packs).

Protein Precipitation in Acetone Protocol

Top Down

Reagents required in this protocol: sodium chloride, acetone

- Screw a Plug onto the base of the Filtration Cartridge.
- If your sample contains no sodium chloride, add NaCl to give a final concentration of 20 to 100 mM.
- Transfer 100 µL of the salted protein to the plugged Filtration Cartridge.
- Add 400 µL room temperature acetone.
- Cap the Filtration Cartridge and rock gently 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 350 ×g (2000 rpm) ×5 minutes. Discard the flow through solvent.
- Wash the protein pellet with 400 µL acetone. Immediately centrifuge at 350 ×g (2000 rpm) ×2 minutes. Discard the flow through wash solvent.

Proceed immediately to the **Resolubilization of Intact Protein Protocol**.

Resolubilization of Intact Protein Protocol

This procedure applies to samples following solvent precipitation

Reagents required in this protocol: 80% formic acid

- With Plug attached, add 150 µL of cold (-20°C) 80% formic acid in water.
- Cap the Filtration Cartridge, place in freezer for 10 minutes, then vortex or sonicate for 1 minute.
- Add 350 µL 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 OPTIONAL SPE Protocol with the SPE Cartridge accessory (available in some trial packs).

ANTICIPATED RESULTS

While certain solution additives may influence protein recovery, as well as intrinsic protein properties, the following is provided as a guideline for recovery and purification efficiency using the ProTrap XG.

Precipitation Efficiency > 95%

SDS Removal > 99.8%

OPTIONAL– SPE Protein/Peptide Clean-Up Protocol

The maximum amount of protein/peptides to load on the SPE Cartridge is 100 µg.

Reagents required in this protocol for bottom-up workflows: acetonitrile, 0.1 % TFA, 5% acetonitrile/0.1% TFA, 50% acetonitrile/0.1% TFA

Reagents required in this protocol for top-down workflows: acetonitrile, 0.1% TFA, 5% acetonitrile/0.1% TFA, 30% isopropanol/42% formic acid, 40% isopropanol/36% formic acid

- The accessory SPE Cartridge must first be primed using the attached Priming Cartridge. Avoid excessive spinning, a few microliters of the final priming solution left behind is permissible during priming, loading, and wash. The final elution step can be spun to complete dryness.
- PRIME: Add 300 µL acetonitrile and spin through the SPE (2000 ×g (4500 rpm) ×2 minutes). Then add 300 µL 0.1% TFA (v/v) in water; spin (2000 ×g (4500 rpm) ×2 minutes).
- Remove SPE Cartridge from the Priming Cartridge and attach to the base of the Filtration Cartridge containing the protein.
- LOAD: Spin at 800 ×g (3000 rpm) ×5 minutes. Ensure that no more than a few microliters of solvent remains in the Filtration Cartridge, if so spin again. OPTIONAL: Reload the eluent into the SPE Cartridge (800 ×g (3000 rpm) ×5 minutes). This second pass can improve SPE retention.
- WASH: Add 300 µL 5% acetonitrile, 0.1% TFA in water. Spin at 2000 ×g (4500 rpm) ×2 minutes. Discard solvent.
- **ELUTE I: For digested peptides**, add 300 µL of 50% acetonitrile, 0.1% TFA, water. Spin (350 ×g (2000 rpm) ×5 minutes). Retain the eluent.

  OR

  ELUTE II: For intact protein, use 300 µL of 30% isopropanol/42% formic acid/28% water (800 ×g (3000 rpm) ×5 minutes), followed by 300 µL of 40% isopropanol/36 % formic/24% water (800 ×g (3000 rpm) ×5 minutes). Pool eluates.

REFERENCES

Crowell, A.M.; MacLellan, D.L.; Doucette, A.A. "A two-stage spin cartridge for integrated protein precipitation, digestion, and SDS removal in a comparative bottom-up proteomics workflow".

J. Proteomics 2015; 118:140-50. doi: 10.1016/j.jprot.2014.09.030

PRODUCT WARRANTY

Proteoform Scientific Inc. guarantees the quality of this product if used as instructed. Any component of the kit found to be defective shall be replaced free of charge upon return of the defective product. Proteoform Scientific Inc. disclaims any implied warranty of merchantability or fitness for a particular purpose, and in no event shall Proteoform Scientific Inc. be liable for consequent damage.

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