PROTEOFORM SCIENTIFIC

ProTrap XG USER GUIDE

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

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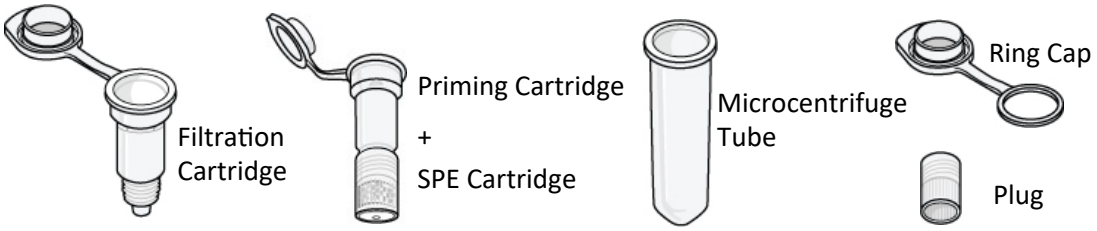
The ProTrap XG is available in three pack sizes and each kit contains the following components:

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



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 SPE Cartridge and Plug screw onto the base of the Filtration Cartridge. To ensure a tight seal, once the components 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 SPE Cartridge or Plug.

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

The following suggested protocols have been optimized using maximum and minimum protein concentrations of 5 mg/mL and 0.01 mg/mL respectively and are provided to demonstrate the potential uses of the ProTrap XG . More dilute protein solutions require extra care, please contact us (info@proteoform.com) for a special protocol.

SUGGESTED PROTOCOLS

- 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 500 µg of protein.

PROTOCOL 1 – Protein Precipitation in Acetone

- Screw a Plug onto the base of the Filtration Cartridge.
- Beginning with your protein sample, add salt (NaCl) to 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.
- Intact proteins may be resolubilized by Protocol 2, or subject to enzymatic digestion within Protocol 3.

PROTOCOL 2 – Resolubilization of Intact Protein

This procedure applies to samples following solvent precipitation in Protocol 1

- 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 SPE (Protocol 4).

PROTOCOL 3 – Protein Digestion in the ProTrap XG

This procedure applies to samples following solvent precipitation in Protocol 1

- 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 SPE cleanup (Protocol 4).

PROTOCOL 4 – SPE Protein/Peptide Clean-Up

- The 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) ×5 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% isoproponal/42% formic acid/28% water (800 ×g (3000 rpm) ×5 minutes), followed by 300 µL of 40% isoproponal/36 % formic/24% water (800 ×g (3000 rpm) ×5 minutes). Pool solvents.

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%

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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PTXG-UG Rev 8/1 202008