ProTrap XG

OPTIONAL SPE Protein/Peptide Clean-Up PROTOCOL

The SPE Cartridge is provided as an OPTIONAL de-salting for your samples. Users should be aware that recovery will be reduced when using the SPE Cartridge.

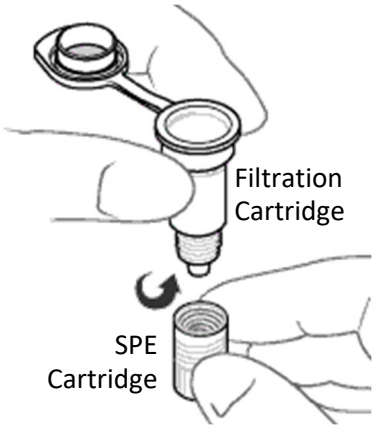
The ProTrap XG device is optimized to process 50 µg of protein using our base protocols. If you have very low amounts or very high amounts of protein, please contact Allumiqs at support@allumiqs.com to discuss protocol adjustments. Additional protocols and the ProTrap XG User Manual are available at allumiqs.com

PREPARATION NOTES

- The maximum amount of protein/peptides to load on the SPE Cartridge is 50 µg.
- **Reagents required in this protocol for bottom-up workflows:**
  100% methanol, 5% acetonitrile/0.1 % TFA, 100% acetonitrile, 50 % acetonitrile/0.1 % TFA
- **Reagents required in this protocol for top-down workflows:**
  100% methanol, 50% acetonitrile/0.1 % TFA, 5% acetonitrile/0.1 % TFA, 100 % acetonitrile, 30% isopropanol/42% formic acid, 40% isopropanol/36% formic acid

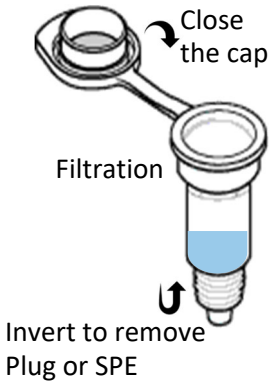
ASSEMBLING THE PROTRAP XG

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

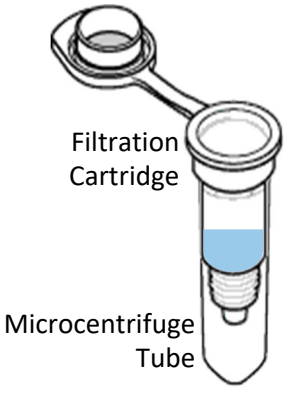


The OPTIONAL SPE screws on to and off of the base of the Filtration Cartridge and Priming Cartridge. To ensure a tight seal, give a firm twist by hand.

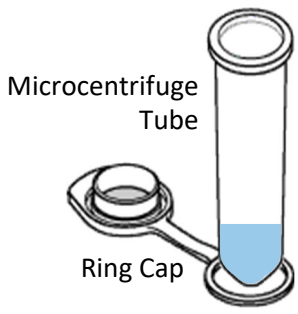
The Plug is attached and removed in the same way.



After sample and reagents have been added to the Filtration Cartridge, cap it and invert before unscrewing the Plug or OPTIONAL 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.

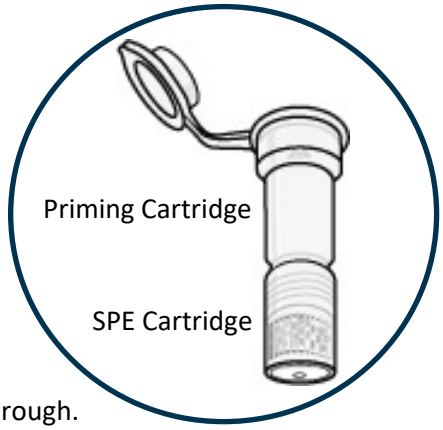
MATERIALS REQUIRED

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

For Bottom-Up Workflows:
methanol
50% acetonitrile/0.1% TFA
5% acetonitrile/0.1% TFA
acetonitrile

For Top-Down Workflows:
methanol
50% acetonitrile/0.1% TFA
5% acetonitrile/0.1% TFA
acetonitrile
30% isopropanol/42% formic acid
40% isopropanol/36% formic acid

SPE PROTEIN/PEPTIDE CLEAN-UP



The SPE Cartridge must first be primed with 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 (Prime the SPE Cartridge just prior to use.)

- Add 600 µL 100% methanol; centrifuge 400 ×g (2000 rpm) ×2 minutes. Discard flow through.
- Followed by 600 µL 5% acetonitrile/0.1% TFA, centrifuge 400 ×g (2000 rpm) ×2 minutes. Discard flow-through.
- Finally, 300 µL 5% acetonitrile/0.1% TFA centrifuge 400 ×g ×1 (2000 rpm) minute. Discard flow-through.
- Remove SPE Cartridge from the Priming Cartridge immediately and attach it to the base of the Filtration Cartridge containing the protein. Place in a clean 2mL tube.

LOAD

- Add 30 µL clean acetonitrile to your sample (bringing the final concentration of acetonitrile to 5%).
- Spin the Filtration Cartridge with the SPE Cartridge attached 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.
- Reload the eluent into the SPE Cartridge 800 ×g (3000 rpm) ×5 minutes. This second pass can improve SPE retention.

WASH

Add 600 µL 5% acetonitrile, 0.1% TFA in water. Spin at 2000 ×g (4500 rpm) ×2 minutes. Discard flow through.

ELUTE

Digested Peptides:

- Add 300 µL of 50% acetonitrile, 0.1% TFA, water. Spin 2500 ×g (5000 rpm) ×5 minutes.
- Retain the eluate.

OR

Intact Protein:

- Add 300 µL of 30% isopropanol/42% formic acid. Centrifuge 2500 ×g (5000 rpm) ×5 minutes.
- Followed by 300 µL of 40% isopropanol/36 % formic acid. Centrifuge 2500 ×g (5000 rpm) ×5 minutes.
- Pool eluates.

Vacuum dry and reconstitute in desired LC-MS/MS solvent.

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