Brainless S-trap Proteomics Protocol Mini Columns or 96 well

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We use this protocol and it's working

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ABSTRACT

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ATTACHMENTS

s-trap-mini-long-4-1-2.pdf

Brainless S-trap Proteomics Protocol Mini Columns or 96 well.xlsx

MATERIALS

Required equipment and materials:
- 1.7 mL sample tubes
- Single channel pipettors with corresponding tips
(P2, P20, P200, P1000)
- Benchtop centrifuge
- pH meter with electrode
- Vortex mixer
- Heat block set to 55 °C
- Water bath or humidified incubator set to 47 °C - SpeedVac or lyophilizer
- Vacuum manifold (optional; Supelco Visiprep
SPE Vacuum Manifold is recommended)
- Sonicator

Required reagents and solutions:
- Protein sample (**100 – 300µg**)
- pH reference solutions
- Tris(2-carboxyethyl)phosphine (**TCEP**)
- Chloroacetamide (**CAA**)
- Protease of choice (e.g. trypsin; 10 µg/column per 100µg of sample)
- Stock of 1 M triethylammonium bicarbonate (**TEAB**), pH 8.5
- Aqueous phosphoric acid
- Sodium deoxycholate (**SDC**)
- LC/MS grade methanol (**MeOH**), formic acid, acetonitrile,
water and isopropanol

Required Solutions - Make 50 ml each ahead of time and store at 4C except trypsin, reducing agent, and alkylator which should be made fresh. Solutions expire in one year.

Solutions	Composition (for 50ml)	pH	Storage
4C Storage			
1X Lysis Buffer	5% SDC (2.5g), 50 mM TEAB (2.5ml of 1M stock), dilute to 50ml with 18.2 MΩ water	8.5	4C
Acidifier, mini/midis (12% phosphoric acid)	Phosphoric acid diluted to 12% with water (7 ml of 85% phosphoric acid diluted into 43 ml)	unadjusted	4C
1M TEAB stock	1M TEAB; pH adjusted with phosphoric acid	7.55	freeze 1.5ml aliquots in 15 ml falcon tubes
Binding/wash buffer	100 mM TEAB (final) in 90% methanol (dilute 5ml of above 1M teab with 45ml MeOH	7.55	4C
Digestion Buffer	50 mM TEAB	8.5	4C
Make Fresh			
Reductant	5mM TCEP in 1x lysis buffer (for 10x sol'n, add 100 ul lysis buffer to 14 mg TCEP, for 1x add 10ul to 1x lysis buffer)	Fresh	
Alkylator	10 mM chloroacetamide (CAA) in 1x lysis buffer (for 10x sol'n, add 100 ul lysis buffer to 10 mg TCEP, for 1x add 10ul to 1x lysis buffer)	Fresh	
Trypsin	Resuspend in 50 mM TEAB per desired concentration, no less than 10 ug trypsin per mini column		

Solubilize In SDS, reduce, alkylate

1 Make up solutions in the materials section ahead of time (at least day before). Trypsin, reducing agent, and alkylator should be made fresh before necessary step.

2 Add 50 µL 1X lysis buffer with reductant and alkylator (heated to 99C) to tissue or pelleted cells. Tissue/cells should be greater than 300 ug.

Note

See number of phosphopeptides detected vs starting protein amount
https://link.springer.com/protocol/10.1007/978-1-4939-3049-4_17/figures/1

3 Boil on heat block (99C) for 10 min.

4 Sonicate with micro tip probe for 15s with pulses of 5 s on and 1 s off at an amplitude of 50 %. Alternatively use bead homogenizer if tissue is too big/tough for sonication. Sonication may be preferred for small tissue pieces.
**If the sample is viscous, sheer DNA thoroughly via sonication or treatment with Benzonase. This is essential as DNA can clog the S-Trap column.*

5 Clarify sample of debris by centrifugation (5 min at 13,000 g to pellet debris). Transfer 50 ul supernatant to new tube or plate.

6 Measure protein concentration with BCA. Make sure final sample has 1-300 ug protein in 50 ul of 1x lysis buffer.
**100 ug protein is more than sufficient for general proteome. 300 ug required for phosphoproteomics.*

7 Add 5 ul acidifier to 50 ul sample (final concentration ~1.1% phosphoric acid). Vortex.

Trap Protein In Column

8 Add 350 ul binding/wash buffer to the sample and mix.

9 Place S-trap mini column into a 2 ml receiver tube for waste flow through. For S-trap 96 well plate, make sure there is waste plate underneath. Using pipette, transfer to S-trap column or 96 well plate. **Do not centrifuge yet.**
*For larger initial volumes, can load column multiple times with volumes < 600ul.

10 Centrifuge S-trap column at 4000 g for 30s to trap proteins (vaccum manifold or 1500g for 2 min for 96 well plate).
*Can also use negative pressure via vacuum manifold or positive pressure from above.
*Clogged columns may be centrifuged as high as 15,000g.

Clean Protein

11 Add 400 ul binding/wash buffer; centrifuge 4000g for 30s (vaccum manifold or 1500g for 2 min for 96 well plate). Repeat 3x and discard flow through as necessary.
*Can rotate columns 180 degrees between centrifugations to ensure homogenous flow.

12 Centrifuge S-trap column at 4000g for 1 min (vaccum manifold or 1500g for 2 min for 96 well plate) to fully remove binding/wash buffer. Failure to do so may result in dripping.

13 Transfer S-trap mini column to a clean 2 ml sample tube or new 96 well receiver plate for digestion.

Incubate and digest protein

14 Add 125 ul digestion buffer containing trypsin at 1:10 wt:wt. It is okay for sample ranges from 1-300 ug to just apply 10 ug trypsin in 125 ul digestion buffer.
*Make sure there are no air bubbles on top of the trap because that prevents sample from entering trap and being properly digestion. Can use tricks to try and break up bubbles if any are present.

15 Cap the s-trap loosely to limit evaporative loss. Cannot be air tight. Incubate for 1-2 hrs at 47C for trypsin or overnight at 37C. Water bath is best since the humid environment limits evaporative loss. If using dry incubator, put a beaker of water in there to humidify. Do not shake.

Elution

16 Add 80 ul elution buffer 1 to S-trap and then centrifuge (4000 rcf for 1 min), (vaccum manifold or 1500g for 2 min for 96 well plate).
*Do not centrifuge the digestion through before applying elution buffer 1. Apply elution buffer 1 directly into the trap containing the digestion buffer that was incubated.

17 Add 80 ul of elution buffer 2 to S-trap then centrifuge (4000 rcf, 1 min), (vaccum manifold or 1500g for 2 min for 96 well plate).

18 Add 80 ul elution buffer 3 to S-trap then centrifuge (4000 rcf, 1 min), (vaccum manifold or 1500g for 2 min for 96 well plate).
*This elution assists in recovery of hydrophobic peptides. Other organics may also be used as needed.

19 Pool elutions. Measure peptide concentrations using pierce quantitative colorimetric peptide assay. Transfer to 96 well axygen PCR microplate if ready to inject in mass-spec. Dry plate in speed vac 2h-overnight. Can also dry down and freeze sample in plate or tube. Dry peptides are a stable stop point.

20 If proceeding to phosphoenrichment, optionally save 50 ug peptides for bulk proteomics and proceed with phosphoenrichment with remaining 250 ug peptides.