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We use this protocol and it's working

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Protein Digestion with S-trap Spin Columns

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ABSTRACT

Trypsin digestion of isolated proteins using S-trap Spin columns in preparation for downstream proteomic profiling.

MATERIALS

- S-trap spin columns (Protifi)
- Centrifuge
- Centrifugal vacuum concentrator
- Small hot box/incubator
- HPLC-grade water
- 10% SDS solution
- 1 M triethylamonium bicarbonate (TEAB) solution, pH 8
- 100 mM triethylamonium bicarbonate (TEAB) solution, pH 8 in water
- Dithiothreitol (DTT; 250 mM in 100 mM TEAB, pH 8)

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- Iodoacetamide (IAA; 250 mM in 100 mM TEAB, pH 8)
- S-trap buffer (90% methanol in 100 mM TEAB)
- Sequencing-grade trypsin
- 12% phosphoric acid in water
- Digestion buffer/Elution buffer 1: 50 mM triethylamonium bicarbonate (TEAB) solution, pH 8 in water
- Elution buffer 2: 0.5% formic acid (FA) in water
- Elution buffer 3: 50% acetonitrile (ACN), 0.5% formic acid (FA) in water
- 0.2% formic acid (FA) in water

1 In a 2-mL microcentrifuge tube add up to 300 µg of the protein lysate, 10% SDS for a final concentration of 4% SDS, 1M TEAB pH 8 solution for a final concentration of 50 mM TEAB, and HPLC-grade water if necessary to bring the volume up to a minimum of 50 µL.

2 Add 250 mM DTT for a final concentration of 20 mM and incubate for 10min at 50°C to reduce the proteins. Then immediately leave for 10 min on the bench at room temperature (RT).

3 Add 250 mM IAA for a final concentration of 40 mM and incubate for 30min at RT in the dark to alkylate the proteins.

4 Acidify the sample with 12% phosphoric acid for a final concentration of 1.2%.

5 Add 7 volumes of S-Trap buffer to the acidified lysate and mix immediately by inversion. Formation of protein colloid may be observed.

6 Use S-trap micro spin columns for ≤ 100 µg of protein or S-trap mini spin columns for 100-300 µg of protein. Ensure that the S-Trap spin column is in a 2.0-mL flow-through catch tube.

7 Add 100 µL (S-trap micro spin column) or 200 µL (S-trap mini spin column) of the acidified lysate/S-Trap buffer mix into the S-Trap spin column. Centrifuge at 4,000 x *g* for 10 seconds or until all the solution has passed through the column. Discard the flow-though.

8 Repeat step 7 until the entire acidified lysate and S-Trap buffer mix has passed through the column.

9 Add 200 µL (S-trap micro spin column) or 400 µL (S-trap mini spin column) of S-Trap buffer to wash the column. Centrifuge at 4,000 x *g* for 10 seconds or until all solution has passed through the column. Discard the wash solution.

10 Add 200 µL (S-trap micro spin column) or 400 µL (S-trap mini spin column) of S-Trap buffer and set aside.

11 Prepare a 1:25 (wt/wt) trypsin/protein ratio solution of sequencing-grade trypsin.

12 Centrifuge the S-trap spin column at 4,000 x *g* for 10 seconds or until the column is dry.

13 Place the S-Trap spin column into a clean 2.0-mL elution tube.

14 Add 125 µL of the sequencing-grade trypsin solution to the column at a 1:25 (wt/wt) trypsin/protein ratio. Be careful not to pierce the trap material.

15 Loosely cap the S-trap micro spin column or close the S-trap mini spin column and incubate for 1 hour at 47°C with no agitation.

16 After 1 hour, add another 125 µL of the sequencing-grade trypsin solution to the column and incubate overnight at 37°C with no agitation.

17 After overnight incubation take the samples out of the incubator and elute sequentially in the same 2.0-mL elution tube as follows:

17.1 a. Add 80 µL of elution buffer 1 (50 mM TEAB, pH 8) and centrifuge at 1,000 x *g* for 1 minute.

17.2 b. Add 80 µL of elution buffer 2 (0.5% FA) and centrifuge at 1,000 x *g* for 1 minute.

17.3 c. Add 80 µL of elution buffer 3 (50% ACN, 0.5% FA) and centrifuge at 4,000 x *g* for 1 minute.

18 Vacuum dry the eluted peptide solution in a centrifugal vacuum concentrator.

19 Reconstitute the dried peptides in 0.2% FA and thoroughly mix the solution before desalting.