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Protocol status: Working
We use this protocol and it's working

Protein Digestion with S-trap Spin Columns using Conditioned Concentrated Media

Forked from 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.

For trypsin digestion of proteins from conditioned media Step 1 is modified as we add a volume of media rather than a concentration of protein homogenate. Step 6 is modified because we use S-trap micro spin columns as conditioned concentrated media samples contain ≤ 100 µg of protein. In step 14 and 16 we use 2 µg sequencing-grade trypsin instead of the usual 1:25 (wt/wt) trypsin/protein ratio.

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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)
- 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 an appropriate proportion of volume from the concentrated condition media, 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 as conditioned concentrated media samples contain ≤ 100 µg of protein. Ensure that the S-Trap spin column is in a 2.0-mL flow-through catch tube.

7 Add 100 µL 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 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 of S-Trap buffer and set aside.

11 Prepare the 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 2 µg of the sequencing-grade trypsin solution to the column. 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 2 µg 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.