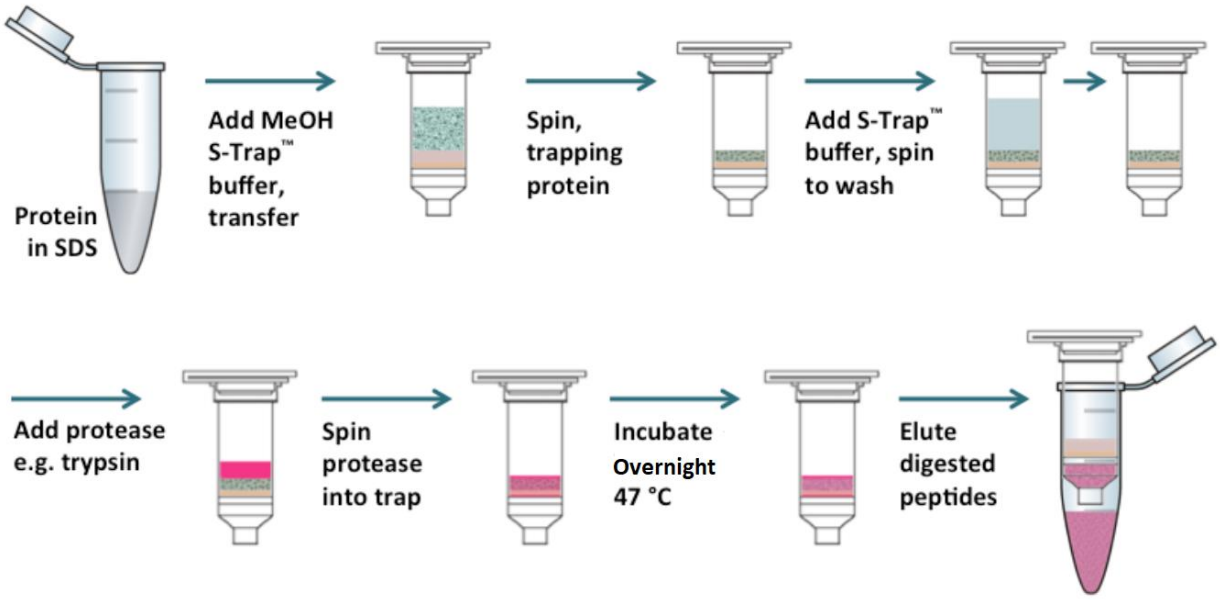
PSR Protocol for S-Trap micro spin column digestion

Ref: https://www.protifi.com/resources/



Reagents

Sodium DodecylSulfate (SDS): Reagent plus, approx. 98.5%(GC), Sigma-Aldrich, Catlg # L4509-25G, MW 288.38

Triethyl Ammonium Bicarbonate (TEAB) buffer: 1M, 50ml: Thermo Scientific, Catlg # 90114

Formic Acid: Optima LC/MS, Fisher Scientific, Catg# A117-50, 50ml, FW 46.02

o-Phosphoric acid, 85% (H3PO4): Certified ACS, Fisher Chemical, Catlg#A242-500,Lot160391 (OHSU Chem. Stores,$53.40,9/5/19), MW 98

Dithiothretol (DTT): Invitrogen, UltraPure, Ctlg#15508-013, MW 154.25

Alpha-Iodoacetamide (IAA): Sigma, Catlg# 16125-10G, MW 185

HPLC grade Water: Fisher Scientific, Optima LC/MS, Ctlg# W6-1

Methanol: Fisher Scientific, Ctlg# A452-4

Trypsin: Promega Sequencing grade,Modified porcine trypsin, Ctlg# V5111, 5x20ug

Solutions

Note: All solutions should be made fresh on day of digestion

1X SDS protein solubilization buffer: 5% SDS, 50mM TEAB (pH7.5). Dissolve 0.25g of SDS in 4ml of HPLC water. Add 0.25ml of 1M TEAB and make up to 5ml with HPLC water.

500mM DTT: Dissolve 18.6mg of DTT in 500uL of HPLC grade water.

500mM IAA: Dissolve 46.3mg of IAA in 500ul of HPLC.

12% Phosphoric acid: Dilute 1.42ml of 85% o-Phosphoric acid to 10ml with HPLC water.

S-Trap Protein binding buffer: 90% aqueous methanol containing final concn. of 100mM TEAB. 9ml of Methanol + 1ml of 1M TEAB. Total volume 10ml.

Digestion Buffer: 50mM TEAB, pH 8. Dilute 0.25ml 1M TEAB to 5ml w HPLC water. Total 5ml.

50mM TEAB: Dilute 500ul of 1M TEAB to 10ml with HPLC water (Store at 4C).

0.2% Formic acid: Add 2ul of Formic acid, Optima LC/MS to 998ul of HPLC water, Optima LC/MS. Total volume 1ml.

50% ACN w 0.2% Formic acid: Add 2ul of Formic acid, Optima LC/MS to 500ul of Acetonitrile and 448ul of HPLC water (prep. 02/14/20)

Trypsin Stock: 80ng/ul in 50mM TEAB. Dissolve 20ug lyophilized trypsin in 250ul of 50mM TEAB (prep. 02/13/20).

50% Methanol: Add 2ml of methanol to 2ml of HPLC water. Total volume 4ml. (prep. 02/14/20)

LC/MS Peptide Dissolution Buffer: 5%FA/3%ACN in HPLC water.Take 0.5ml of Formic acid,0.3ml of Acetonitrile and make up to 10ml wHPLC water (Not prepared).

Equipment

Eppendorf Bench-top centrifuge 5430R

Eppendorf ThermoMixer C, initially set to 47C in MRB524

Thermo Savant Speedvac in MRB Rm 521

Eppendorf Protein LoBind tubes, 0.5ml, Ctlg# 022431064, 1.5ml, Ctlg# 022431081

Fisher Scientific Digital Vortex Mixer

HYBAID Incubator in MRB Rm524

Protocol

1) Aliquot 1-100ug of each sample into 1.5ml Lobind tubes.

2) Dry samples in speedvac for 2h.

3) Dissolve sample in 75ul of 1X SDS protein solubilization buffer (5%SDS, 50mM TEAB) and vortex for 30sec.

5) Centrifuge samples for 3min in Eppendorf 5430R centrifuge at 18300xg.

6) Reduce samples by adding 3.4ul of 500mM DTT and incubate at 95C for 10min in Eppendorf ThermoMixer C. Final concn. of DTT is 22mM in total volume of 78.4ul.

7) Cool samples to room temp. for 10min.

8) Alkylate samples by adding 6.8ul of 500mM IAA to each sample and incubate at room temp in the dark for 30min. Final concn. of IAA is 40mM in total volume of 85.2ul.

9) Add 8.5ul of 12% aqueous phosphoric acid (1:10) for a final concentration of ~1.2% phosphoric acid (i.e. 8.5ul into 85.2ul sample). Mix. Total volume 93.7ul.

Note: This step is essential as the protein trap binds at this pH.

10) Add 562ul (six times total volume) of S-Trap protein binding buffer (90% aqueous methanol containing 100mM TEAB, pH7.5). Mix. Total volume 656ul.

Note1: Volumes of S-Trap buffer from 6-9x of the acidified SDS protein solution are acceptable.

Note2: Colloidal protein particulate is instantly formed in this step. Given sufficient protein, the solution will appear translucent.

12) Transfer S-Trap micro to a 1.5ml lobind tube to collect the flow-through.

13) Add 164ul of the acidified SDS lysate/MeOH S-Trap buffer mixture into the micro column. No column preequilibration is necessary.

Note: Do not add more solution than will fit in the narrow "stem" of the spin column. If initial SDS lysate volume is higher, load the column multiple times until the full volume has been bound (Ex. For 75ul initial sample volume and 656ul final volume, pass 164ul through four times)

14) Centrifuge the micro column at 4,000 g until all SDS lysate/S-Trap buffer has passed through the S-Trap column. Centrifuged for 3min.

Note1: Protein will be trapped within the protein-trapping matrix of the spin column. Vacuum from below or pressure from above can also be used as desired.

Note2: Unsheered DNA, highly viscous proteins (e.g. from mucosal membranes) or spin column overloading may necessitate significantly longer spin times. Do not exceed 5,000xg with the S-Trap micro or mini spin columns.

15) Wash captured protein by adding 150ul of S-Trap protein binding buffer (90% aqueous methanol w 100mM TEAB) and centrifuging at 4,000 g for 3min. Repeat wash three times.

For best results, rotate the S-Trap micro units like a screw 180 degrees between the centrifugations of steps 14 and 15 especially if using a fixed-angle rotor.

Use a simple mark on the outside edge + "tab out" then "tab in". More than three washes can be performed if contamination is observed by mass spectrometry.

Note: Do six washes to completely remove SDS as it inhibits digestion as well as contaminates the mass spectrometer.

16) Move S-trap micro column to a clean 1.5ml lobind tube.

17) Add 40ul of digestion buffer i.e. 80ng/ul trypsin (3.2ug) in 50mM TEAB to each sample (1:16 enzyme:substrate ratio) into the top of the micro column.

Note1: Ensure there is no air bubble between the protease digestion solution and the protein trap. Do not apply less than 0.75ug of trypsin/column. The protein trap is hydrophilic and will absorb the solution.

Note2: As with all digestions, optimization of amount of trypsin, digestion time, buffer and temperature is sample- and enzyme-dependent. Optimize as necessary. Mass spec compatible detergents such as Rapigest and ProteaseMax are compatible with the S-

Trap sample digestion and for some sample types have been observed to aid in digestion and sample recovery.

18) Cap the S-Trap tip column loosely to limit evaporative loss.

Note: Do not make an airtight seal with the cap. If an airtight seal is present, as the heat of incubation expands the air in the headspace above the trap, the increased pressure will force the digestion solution out of the column.

19) Incubate in the incubator at 47degC overnight (inMRB524). Do not shake. Ensure the entire column is exposed to heat. Put a beaker of hot water in the incubator 4-5h before start of incubation to humidify the chamber and prevent the S-Trap column from drying out.

20) Since the S-Trap micro might have dried out during the overnight incubation, add 20ul of digestion buffer (50mM TEAB) to rehydrate each sample and incubate at 37C for 15min.

21) Elute the peptides with 40ul each of 50mM TEAB and then 0.2% aqueous formic acid. Centrifuge elution's through at 4,000g for 4min. Recover hydrophobic peptides with elution of 35ul 50% Acetonitrile containing 0.2% formic acid. Pool elutions.

22) Dry down eluted peptides in a speedvac at room temp for 1.5h.

23) Dissolve dried peptides in 100ul of 50% Methanol (prep. 9/9/19), vortex and evaporate in speedvac for 2h.

Note: Step23 was an additional step added by Ashok to remove any remaining TEAB in the samples as TEAB interferes with the peptide assay.

24) Store samples at -80C or proceed to peptide assay.