S-Trap sample processing: serum and plasma

S-Trap sample processing allows serum and plasma samples to be reproducibly digested even for difficult cases such as samples from patients with liver failure or hyperlipidemia. Sodium dodecyl sulfate (SDS), which S-Trap sample processing is based upon, denatures and solubilized all proteins including hydrophobic lipoproteins. SDS also strips lipids from the proteins of fatty biological samples, resulting in clean peptides. Finally, SDS allows for processing at high protein concentrations (including during reduction and alkylation) so peptides can be eluted at high concentrations, speeding binding in enrichments such as SISCAPA.

This protocol is scaled to yield ~300 µg of digested serum or plasma. Note that other S-Trap sizes can be used for larger or smaller quantities of protein. S-Trap micros have maximum capacity of 100 µg, the maximum for minis is 300 µg and midis can be used from 300 µg to several mg.

Protocol: mini and 96-well plate

1) Quantitative experiments must match amount samples either at the level of protein or peptides (and preferably both). Thus, first determine protein concentration of the plasma or sample (BCA, Bradford, etc.). If a protein assay is not run before sample digestion, peptide levels must be matched after digestion (e.g. use 3-(4carboxybenzoyl)quinoline- 2-carboxaldehyde (CBQCA) or *o*-phthalaldehyde (OPA) assays).
2) Calculate the amount required for 300 µg. As serum and plasma are typically at ~80 mg/mL, this will be approximately **3.75 µL**.
3) Dilute the ~3.75 µL plasma or serum into 40 µL of 1X 5% SDS lysis buffer (white cap in the ProtiFi S-Trap Universal MS Sample Prep Kit; note the kit comes with 2X buffer).
4) Reduce proteins by adding 2 µL of 115 mM TCEP (red cap in the ProtiFi S-Trap Universal MS Sample Prep Kit). The final concentration will be 5 mM. Incubate 10 min at 37 °C (55 °C can be used if desired). The presence of SDS prevents protein precipitation of the disulfide rich blood proteins.
5) Add 4 µL of 187.5 mM MMTS (yellow cap in the ProtiFi S-Trap Universal MS Sample Prep Kit). Incubate at room temperature for 10 min.
6) Add 5 µL 12% phosphoric acid to the 50 µL of SDS solubilized, reduced and alkylated sample (green cap in the ProtiFi S-Trap Universal MS Sample Prep Kit; make sure to first add methanol).
7) Add 350 µL of S-Trap binding buffer (90% MeOH, 100 mM TEAB final; pH 7.1) to the acidified lysate (large bottles in the ProtiFi S-Trap Universal MS Sample Prep Kit; make sure to add methanol first).
8) Add the acidified SDS lysate/S-Trap buffer mix into the spin column.
9) Spin in bench-top centrifuge for 30 s at 4,000 g or until all solution has passed through. Protein will be bound within the protein trap. Remove flow through.
10) Wash the trapped protein by adding 400 µL S-Trap binding buffer to the spin column and centrifuging through. Repeat three times. Remove flow through. Perform the washes in one 2 mL tube, then transfer the spin column to one of the included fresh 2 mL tubes. This prevents contamination of the digestion.

11) Add trypsin at 1:10 – 1:25 wt:wt in 125 µL of 50 mM TEAB, pH 8. This buffer without enzyme is in the blue caped tubes in the ProtiFi S-Trap Universal MS Sample Prep Kit. Spin protease into column briefly; return any solution that passes through to the top of the column. The protein-trapping matrix is highly hydrophilic and will absorb the solution. However, ensure there is no bubble atop the protein trap. Flick if needed.
12) Cap the spin column and incubate in a clean tube for ≥ 1 hr at 47 °C (for trypsin). Most preferably use a water bath. DO NOT SHAKE. Shaking will impede performance. Some dripping is harmless.
13) Elute peptides with 80 µL each of 50 mM TEAB and then 0.2% aqueous formic acid. Add the TEAB elution to the trap with protease prior to centrifugation. Centrifuge elutions at 1,000 g.
14) Elute hydrophobic peptides with 80 µL of 50% acetonitrile, 0.2% formic acid.
15) Dry down peptides. Especially for quantitative work, if protein concentrations have not been matched before hand, peptide levels must be matched after digestion.
16) Resuspend as desired (buffer A or MALDI matrix) and analyze.