S-Trap™ Micro High Recovery Protocol

1) Elute protein from IPs or dissolve protein in 25 µL of high recovery urea-SDS lysis/ solublization buffer: 5% SDS, 8 M urea, 100 mM glycine* pH 7.55.
2) Reduce by adding 1 µL reductant (120 mM aq. TCEP). Incubate at 37 °C for 15 min.
3) Alkylate by adding 1 uL alkylator (500 mM MMTS). Incubate at RT for 15 min.
4) Add 2.5 µL **55% aq. phosphoric acid** to the 25 µL sample. This is different than the normal protocol.
5) Add 165 µL of S-Trap binding buffer (90% MeOH, 100 mM final TEAB, pH 7.55) into the S-Trap micro column. It will not flow through.
6) The next two steps must be done as quickly as possible. Add 2 µg of trypsin/lys-C mix into the acidified sample, immediately mix by pipetting up and down, then immediately transfer the mixture into the S-Trap binding buffer held in the micro spin column. Again mix by pipetting up and down.
7) Spin in bench-top centrifuge in a standard 1.7 mL sample tube at 4,000 g until all solution has passed through. Remove flow through.
8) Wash by adding 150 µL S-Trap buffer to the spin column and centrifuging through. Remove flow through. Repeat three times. Protein will not be lost during washes.
9) Add 0.5 µg of trypsin in 25 µL of 50 mM TEAB, pH 8 to the top of the protein trap. The S-Trap is highly hydrophilic and will absorb the solution. However, ensure there is no bubble atop the protein trap.
10) Cap the spin column **loosely** and incubate in a clean tube for 2 hrs at 47 °C for trypsin. Incubate in a water-saturated atmosphere. **DO NOT SHAKE. The cap MUST NOT form an air-tight seal.**
11) Elute peptides with 40 µL each of 50 mM TEAB and then 0.2% aqueous formic acid. Add the first TEAB elution to the trypsin solution prior to any centrifugation. Centrifuge elutions through at 4,000 g.
12) Elute hydrophobic peptides with 35 µL 50% acetonitrile, 0.2% formic acid.
13) Lyophilize peptides and resuspend as desired (buffer A or MALDI matrix).

*Glycine will be removed during washing and helps to limit carbamylation from activated urea.

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