S-Trap™ Micro Ultra-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. TEAB can also be used.*
2) Clarify sample as needed by centrifugation for 8 min at 13,000 g.
3) Reduce and alkylate disulfides. Avoid high temperatures are not recommended due to urea.
4) Add 2.5 µL 12% phosphoric acid to the lysate solubilized in 25 µL SDS buffer.
5) Add 165 µL of S-Trap binding buffer (90% MeOH, 100 mM final TEAB, pH 7.1) into the S-Trap micro column. It will not flow through.
6) The next two steps must be done as quickly as possible. Add 1 or 2 µg of trypsin to the acidified SDS and immediately mix by pipetting up and down, then immediately transfer the acidified lysate plus trypsin into the S-Trap binding buffer within the 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 protein trapping matrix 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 1 hr at 47 °C for trypsin. Most preferably use a water bath or thermomixer. **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) Dry down peptides and resuspend as desired (buffer A or MALDI matrix).

*Glycine will be removed during washing and is preferable to TEAB to limit carbamylation from urea.

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