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S-Trap™ Micro Spin Column High Recovery

S-Trap™ Micro Spin Column High Recovery allows for the solubilization, reduction, alkylation, and digestion of **1 µg - 10 µg of total protein** cleaned and ready for peptide analysis.

Additional Reagents/Solutions (Not Included)

Liquid Chromatography-Mass Spectrometry (LC-MS) grade is recommended, where applicable

- Protein sample **(1 µg - 10 µg of total protein)**
- Protease of choice (e.g. Trypsin or Trypsin/Lys-C)*
- LC-MS grade Water
- LC-MS grade Isopropyl Alcohol (IPA)
- LC-MS grade Methanol (MeOH)
- LC-MS grade Acetonitrile (ACN)
- LC-MS grade Formic Acid (FA)
- SDS
- 1 M Triethylammonium Bicarbonate (TEAB), pH 8.5
- (Tris(2-carboxyethyl)phosphine) (TCEP)
- Methyl Methanethiosulfonate (MMTS)
- Phosphoric Acid
- Benzonase® (optional)*
- Urea
- Glycine

*** Benzonase® and proteases are susceptible to freeze-thaw cycles.**

Equipment/Materials (Not Included)

- pH meter with reference solutions
- Balance
- Single channel pipettes (P2 through P1000) and corresponding tips
- 1.7 mL sample tubes
- Benchtop centrifuge
- Vortex mixer
- Heat block (adjusted to 55 °C)
- pH paper (pH < 1, optional)
- Water bath or humidified incubator (adjusted to 47 °C)
- Lyophilizer or SpeedVac
- Positive pressure apparatus or vacuum manifold (optional)
- Sonicator (optional, recommended)

Stock Solutions and Reagent to Prepare PRIOR to Start:
All indicated solutions (**) can be purchased at protifi.com

Protein Sample	Your sample	1 µg – 10 µg
Protease	Your choice of protease (E.g., Trypsin or Trypsin/Lys-C)	1 µg - 10 µg per 100 µg of sample in **Solution 6 (Digestion Solution)**
Benzonase (optional)	Benzonase® and $MgCl_2$	Benzonase & 50 mM $MgCl_2$
Solution 1**	High Recovery Lysis Solution	5% (w/v) SDS, 8 M urea, 100 mM glycine in LC-MS grade Water, pH 7.55
Solution 2**	Reductant	120 mM TCEP in LC-MS grade Water
Solution 3**	Alkylator	500 mM MMTS in LC-MS grade IPA (mass shift is + 45.988, addition of SCH_2)
Solution 4**	Acidifier	55.0% (v/v) Phosphoric Acid in LC-MS grade Water*
Solution 5**	Binding/Wash Solution	100 mM TEAB (final) in 90% LC-MS grade MeOH, pH 7.5
Solution 6**	Digestion Solution	50 mM TEAB in LC-MS grade Water, pH 8.0
Solution 7 (optional)**	Elution Solution	0.2% (v/v) LC-MS grade FA in LC-MS grade Water
Solution 8 (optional)	Elution Solution 2	50% (v/v) LC-MS grade ACN in 0.2% (v/v) LC-MS grade FA

All solutions can be made in advance and stored at -20 °C or colder. After thawing, mix thoroughly to ensure any precipitants are redissolved.

**** Acidifier must be stored tightly capped; it will degrade upon atmospheric exposure.***

RT = room temperature (20-25 °C), v/v = volume-to-volume ratio, w/v = weight-to-volume ratio, w/w = weight-to-weight ratio

Protocol:

Refer to Appendices for Sample-Specific Considerations, Alternative Proteases, and Troubleshooting Tips.

1. **Denature Protein.** Elute protein from immunoprecipitations or dissolve protein in 25.0 µL of **Solution 1 (High Recovery Lysis Solution)**.
2. If sample is viscous due to the presence of DNA, sheer it thoroughly by probe sonication or enzymatically with a nuclease such as Benzonase™ (see Appendix D). Unsheared DNA will clog the protein trap.
3. Clarify sample as desired by centrifugation (e.g. 13,000 x g for 8 minutes). Transfer clarified lysate to tubes. Pellet can be analyzed separately (see Appendix D).
4. **Reduce.** Add 1.0 µL of **Solution 2 (Reductant)**. Vortex briefly and incubate at 55 °C for 15 minutes.
5. **Alkylate.** Add 1.0 µL of **Solution 3 (Alkylator)**. Vortex briefly and incubate at RT for 10 minutes.
6. **Acidify.** Add 2.5 µL of **Solution 4 (Acidifier)** to the sample. Vortex briefly. pH paper can be used to ensure pH ≤ 1. Proceed to the next steps immediately.
7. Place an **S-Trap™ Micro Spin Column** in a 1.7 mL sample tube for waste flow-through.
8. Add 165 µL of **Solution 5 (Binding/Wash Solution)** to the **S-Trap™ Micro Spin Column**.
9. Quickly, add 0.5 µg of trypsin or trypsin/Lys-C to the acidified sample. Mix by pipetting.
10. Immediately transfer the sample with trypsin or trypsin/Lys-C to the **S-Trap™ Micro Spin Column**. Mix by pipetting.
    Note: *The sample may appear translucent at this step due to colloidal protein formation.*
11. **Trap Protein.** Centrifuge the **S-Trap™ Micro Spin Column** and waste flow-through tube at 10,000 x g for 30 seconds. Visually confirm that all solution passed through the column. If not, centrifuge again until no liquid remains. If the solution still hasn't passed through, the column may be centrifuged as high as 15,000 x g until all solution passed through. Discard flow-through and return the column to the 1.7 mL sample tube.
    Note: *The S-Trap™ Micro Spin Columns end in a Luer taper. A vacuum manifold or positive pressure can be us to draw solution through the column.*
12. **Clean Protein.** Add 150 µL of **Solution 5 (Binding/Wash Solution) with MeOH added** to the **S-Trap™ Micro Spin Column** and centrifuge at 10,000 x g for 30 seconds. Visually confirm that all solution passed through the column. Repeat washes 3 times; discard flow-through as necessary. Return the column to the 1.7 mL sample tube.
    Note: *For best results, rotate the column 180° between centrifugations. Marking the outside edge of t make sit easier to track rotations.*
13. Centrifuge the **S-Trap™ Micro Spin Column** at 10,000 x g for 1 minute to fully remove all Binding/Wash Solution.
14. Transfer the protein-containing **S-Trap™ Micro Spin Column** to a clean 1.7 mL sample tube for digestion.
15. Dilute trypsin or trypsin/Lys-C in **Solution 6 (Digestion Solution)** to a final volume of 20.0 µL, ensuring that the amount of trypsin in this solution is 0.2 µg.
16. Transfer the entire 20.0 µL of trypsin or trypsin/Lys-C solution to the **S-Trap™ Micro Spin Column**. The matrix is hydrophilic and will absorb the solution; no centrifugation is necessary.
    Note: *If bubbles are present, flick the tube gently to remove them and/or spin the column very brie benchtop centrifuge. If any solution flows through, pipette it back on top of the column.*
17. **Incubate & Digest.** Loosely screw the cap on the **S-Trap™ Micro Spin Column** to limit evaporative loss so that trypsin solution is not pushed through the column due to thermal expansion; allow air to escape. Pipette any solution that passes through back on top of the column. Place the column and sample tube in a 47 °C water bath or humidified incubator for 2 hours. If using a water bath, make sure the column matrix sits below the water level to ensure even heating.
18. **Elute 1.** Remove the **S-Trap™ Micro Spin Column** with sample tube from the incubator and add 40.0 µL of **Solution 6 (Digestion Solution)** to the top of the column. Centrifuge at 10,000 x g for 1 minute.
19. **Elute 2.** Add 40.0 µL of **Solution 7 (Elution Solution)** to the top of the **S-Trap™ Micro Spin Column**. Centrifuge at 10,000 x g for 1 minute.
20. **Elute 3.** If hydrophobic peptides are of interest, add 40.0 µL **Solution 8 (Elution Solution 2)** to the top of the **S-Trap™ Micro Spin Column**. Centrifuge at 10,000 x g for 1 minute.
21. Lyophilize or SpeedVac eluted peptides collected from Steps 18-20 in the sample tube. Resuspend as needed for subsequent analysis (e.g. Aqueous Buffer A such as 5% ACN, 0.1% FA).