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S-Trap™ 96-well Micro Kit

The ProtiFi™ **S-Trap™** technology combines robust Sodium Dodecyl Sulfate (SDS) solubilization of biological samples and enhanced contaminant removal for proteomic analysis by mass spectrometry. The **S-Trap™ 96-well Micro Kit** provides all the solutions for the solubilization, reduction, alkylation, and digestion of 96 samples containing **≤ 100 µg of total protein**. The kit fully removes a broad range of contaminants incompatible with downstream proteomics analysis, such as detergents, salts, buffers, stabilizers, excipients, and others, while yielding high peptide recovery.

Additional Reagents/Solutions (Not Included)

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

- Protein sample **(1 µg - 100 µg of total protein)**
- Protease of choice (e.g. Trypsin; 1 µg per 10 µg sample)*
- LC-MS grade Water
- LC-MS grade Methanol (MeOH)
- LC-MS grade Acetonitrile (ACN)
- LC-MS grade Formic Acid (FA)
- Benzonase® (optional)*

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

Equipment/Materials (Not Included)

- Single- and/or multi-channel pipettes (P2 through P1000) and corresponding pipette tips
- Centrifuge capable of processing 96-well plates at 2000 x g
- Vortex mixer
- Heat block (adjusted to 55 °C)
- pH paper (pH < 1, optional)
- Humidified incubator (adjusted to 47 °C)
- Lyophilizer or SpeedVac
- Positive pressure apparatus or vacuum manifold (optional)
- Sonicator (optional, recommended)

Contents: (1) S-Trap™ 96-well Micro Plate, (1) 2.0 mL 96-well plate, (1) 1.0 mL 96-well plate, (1) 96-well cover

①	Solution 1	2x Lysis Solution	1 x 1.5 mL	10% (w/v) SDS, 100 mM TEAB in LC-MS grade Water, pH 7.55
②	Solution 2	Reductant	1 x 200 µL	120 mM TCEP in LC-MS grade Water
③	Solution 3	Alkylator	1 x 200 µL	500 mM MMTS in LC-MS grade IPA (mass shift is + 45.988, addition of SCH_2)
④	Solution 4	Acidifier	1 x 550 µL	27.5% (v/v) Phosphoric Acid in LC-MS grade Water*
⑤	Solution 5	Binding/Wash Solution	4 x 3.0 mL (30.0 mL bottles)	100 mM TEAB (final) in 90% LC-MS grade MeOH, pH 8.5 **Note: 27.0 mL LC-MS grade MeOH must be added to each bottle before use**
⑥	Solution 6	Digestion Solution	1 x 7.0 mL	50 mM TEAB in LC-MS grade Water, pH 8.0
⑦	Solution 7	Elution Solution	1 x 9.0 mL	0.2% (v/v) LC-MS grade FA in LC-MS grade Water

All provided solutions, including Binding/Wash Solution with MeOH added, can be stored 1 month at 4 °C or up to 1 year at -20 °C. After thawing, mix thoroughly to ensure any precipitants are redissolved.

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

IPA = isopropyl alcohol, MMTS = methyl methanethiosulfonate, RT = room temperature (20-25 °C), TCEP = (tris(2-carboxyethyl)phosphine), TEAB = triethylammonium bicarbonate, 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 **1.** **Denature Protein.** To 11.5 µL of liquid sample, add 11.5 µL of **Solution 1 (2x Lysis Solution)**. For solid samples, dilute **Solution 1 (2x Lysis Solution)** to 1x by adding an equal volume of LC-MS grade water. Add 23.0 µL of 1x Solution 1 to a solid sample. Sonicate or vortex to fully solubilize and denature proteins. Samples can be processed in tubes or a deep well 96-well plate.

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 or a deep well 96-well plate. Pellet can be analyzed separately (see Appendix D).

② **4.** **Reduce.** Add 1.0 µL of **Solution 2 (Reductant)**. Incubate at 55 °C for 15 minutes.

③ **5.** **Alkylate.** Add 1.0 µL of **Solution 3 (Alkylator)**. Incubate at RT for 10 minutes.

⑤ **6.** While the sample is incubating, add 27.0 mL of LC-MS grade MeOH to a bottle of **Solution 5 (Binding/Wash Solution)**. Mix thoroughly.

④ **7.** **Acidify.** Add 2.5 µL of **Solution 4 (Acidifier)** to the sample. pH paper can be used to ensure pH ≤ 1. Proceed to the next step immediately.

⑤ **8.** Add 165 µL of **Solution 5 (Binding/Wash Solution) with MeOH added** to the sample. Mix thoroughly.
Note: The sample may appear translucent at this step due to colloidal protein formation. Do NOT centrifuge.

9. Place an **S-Trap™ 96-well Micro Plate** atop the 2.0 mL 96-well plate. Transfer each sample, including any insoluble material, into a well of the **S-Trap™ 96-well Micro Plate**.
Note: No pre-equilibration is necessary. Solution may begin to drip through immediately; this is expected. The well can hold ~400 µL of solution. For larger volumes, the well can be loaded multiple times with the solution from Step 8. When transferring into the well, do not disturb the matrix.

10. **Trap Protein.** Centrifuge the **S-Trap™ 96-well Micro Plate** and waste plate at 2,000 x g for 2 minutes. Visually confirm that all solution passed through the well. If not, centrifuge until no liquid remains. Discard flow-through and return the plate to the 2.0 mL 96-well plate.
Note: A vacuum manifold or positive pressure can be used to draw solution through the plate provided that all wells have flow behavior, which might not be the case for all samples.

⑤ **11.** **Clean Protein.** Add 250 µL of **Solution 5 (Binding/Wash Solution)** to the **S-Trap™ 96-well Micro Plate** and centrifuge at 2,000 x g for 2 minutes. Visually confirm that all solution passed through the well. Repeat washes 3 times; discard flow-through as necessary. Return the plate to the 2.0 mL 96-well plate.
Note: Additional wash(es) may be performed if desired. Washes may be captured with the flow-through. A vacuum manifold or positive pressure may also be used if the wells have similar flow behavior.

12. Move the **S-Trap™ 96-well Micro Plate** atop the 1.0 mL 96-well plate for collection.

⑥ **13.** Dilute trypsin in **Solution 6 (Digestion Solution)** to a final volume of 20.0 µL, ensuring that the amount of protease in this solution is at a 1:10 (w/w) ratio with the total amount of protein in the sample (e.g. 10 µg per 100 µg of sample).

14. Slowly and carefully transfer the entire 20.0 µL of trypsin solution to the **S-Trap™ 96-well Micro Plate**. Gently tap the plate a few times and visually compare to an empty well to ensure solution entered the matrix.

⑥ **15.** **Incubate & Digest.** Loosely cover the **S-Trap™ 96-well Micro Plate** to limit evaporative loss without making an air-tight seal. Place the plate atop the 1.0 mL 96-well plate in a 47 °C humidified incubator for 1 hour. After 1 hour, add 12.0 µL of **Solution 6 (Digestion Solution)** to all wells. Loosely cover and place back in a 47 °C humidified incubator for 1 hour.
Note: Some dripping may occur during incubation; this is not of concern. Do NOT shake.

⑥ **16.** **Elute 1.** Add 40.0 µL of **Solution 6 (Digestion Solution)** to all wells of the **S-Trap™ 96-well Micro Plate** atop the 1.0 mL 96-well plate after incubation. Centrifuge at 2,000 x g for 2 minutes or until all solution has passed through.

⑦ **17.** **Elute 2.** Add 40.0 µL of **Solution 7 (Elution Solution)** to all wells of the **S-Trap™ 96-well Micro Plate**. Centrifuge at 2,000 x g for 2 minutes.

18. **Elute 3.** If hydrophobic peptides are of interest, add 40.0 µL of 50% (v/v) LC-MS grade ACN in 0.2% (v/v) LC-MS grade FA (solution not provided) to the top of the **S-Trap™ 96-well Micro Plate**. Centrifuge at 2,000 x g for 2 minutes.

19. Lyophilize or SpeedVac eluted peptides collected from Steps 16-18 in the 1.0 mL 96-well plate. Resuspend as needed for subsequent analysis (e.g. Aqueous Buffer A such as 5% ACN, 0.1% FA).