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S-Trap™ Turbo™ 96-well Mini Kit

The ProtiFi™ **S-Trap™ Turbo™** includes all the benefits of the standard S-Trap™, but eliminates the need to concentrate samples. 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™ Turbo™ 96-well Mini Kit** provides all the solutions for the solubilization, reduction, alkylation, and digestion of 96 samples containing **10 µg - 250 µg 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. Ready-to-shoot peptides are prepared in as few steps as possible – ideal for those time conscious researchers.

Additional Reagents/Solutions (Not Included)

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

- Protein sample **(10 µg - 250 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)
- 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 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™ Turbo™ 96-well Mini 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	2 x 1.5 mL	Strong detergent to solubilize sample
②	Solution 2	Reductant	1 x 200 µL	Reductant to break disulfide bonds
③	Solution 3	Alkylator	1 x 200 µL	Alkylator to prevent disulfides from reforming (mass shift is + 45.988, addition of SCH_2)
④	Solution 4	Acidifier	1 x 550 µL	Acidic solution to lower sample pH*
⑤	Solution 5	Binding/Wash Solution	4 x 3.0 mL (30.0 mL bottles)	Volatile buffer with alcohol to facilitate protein trapping and cleaning **Note: 27.0 mL LC-MS grade MeOH must be added to each bottle before use**
⑥	Solution 6	Digestion Solution	1 x 9.0 mL	MS compatible volatile buffer for optimal digestion and elution

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.***

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

Protocol:

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

① **1.** **Denature Protein.** To 23.0 µL of liquid sample, add 23.0 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 46.0 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 2.0 µL of **Solution 2 (Reductant)**. Incubate at 55 °C for 15 minutes.

③ **5.** **Alkylate.** Add 2.0 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 5.0 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 350 µL of **Solution 5 (Binding/Wash Solution)** 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™ Turbo™ 96-well Mini Plate** atop the 2.0 mL 96-well plate. Transfer each sample, including any insoluble material, into a well of the **S-Trap™ Turbo™ 96-well Mini Plate**.

__Note:__ No pre-equilibration is necessary. Solution may begin to drip through immediately; this is expected. The well can hold ~450 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™ Turbo™ 96-well Mini Plate** and waste plate at 2,000 x g for 2 minutes. Visually confirm that all solution passed through the well. If not, centrifuge again for 5 minutes 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 200 µL of **Solution 5 (Binding/Wash Solution)** to the **S-Trap™ Turbo™ 96-well Mini 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™ Turbo™ 96-well Mini 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 30.0 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).

⑥ **14.** Transfer the entire 30.0 µL of trypsin or trypsin/Lys-C solution to the **S-Trap™ Turbo™ 96-well Mini Plate**, ensuring the matrix is fully covered by digestion solution.

15. **Incubate & Digest.** Loosely cover the **S-Trap™ Turbo™ 96-well Mini 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 2 hours.

__Note:__ Some dripping may occur during incubation; this is not of concern. Do NOT shake. If evaporation is significant during incubation, add 10.0 µL Solution 6 (Digestion Solution).

⑥ **16.** **Elute.** Add 20.0 µL of **Solution 6 (Digestion Solution)** to all wells of the **S-Trap™ Turbo™ 96-well Mini 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.

17. Peptides ready for analysis.