

S-Trap™ 96-well Mini 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 Mini Kit** provides all the solutions for the solubilization, reduction, alkylation, and digestion of 96 samples containing **100 µg - 300 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 (**100 µg - 300 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 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 Mini Plate, (1) 2.0 mL 96-well plate, (1) 1.0 mL 96-well plate, (1) silicone mat

1	Solution 1	2x Lysis Solution	2 x 1.5 mL	10% (w/v) SDS, 100 mM TEAB in LC-MS grade Water, pH 7.55
2	Solution 2	Reducant	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 30.0 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 23.0 μ L of liquid sample, add 23.0 μ L 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 μ L 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 μ L of **Solution 2 (Reducant)**. Incubate at 55 °C for 15 minutes.
- ③ 5. **Alkylate.** Add 2.0 μ L 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 μ L 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 μ L 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 Mini 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 Mini Plate**.

Note: No pre-equilibration is necessary. Solution may begin to drip through immediately; this is expected. The well can hold ~450 μ L 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 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 250 μ L of **Solution 5 (Binding/Wash Solution)** to the **S-Trap™ 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™ 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 125 μ L, 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 μ g per 100 μ g of sample).
14. Transfer the entire 125 μ L of trypsin solution to the **S-Trap™ 96-well Mini Plate**, ensuring the matrix is fully covered by digestion solution.
- ⑥ 15. **Incubate & Digest.** Loosely cover the **S-Trap™ 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 1 hour. After 1 hour, add 80.0 μ L 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 80.0 μ L of **Solution 6 (Digestion Solution)** to all wells of the **S-Trap™ 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.
- ⑦ 17. **Elute 2.** Add 80.0 μ L of **Solution 7 (Elution Solution)** to all wells of the **S-Trap™ 96-well Mini Plate**. Centrifuge at 2,000 x g for 2 minutes.
18. **Elute 3.** If hydrophobic peptides are of interest, add 80.0 μ L of 50% (v/v) LC-MS grade ACN in 0.2% (v/v) LC-MS grade FA (solution not provided) to all wells of the **S-Trap™ 96-well Mini 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).