

S-Trap™ 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™ Micro Kit** provides all the solutions needed for the solubilization, reduction, alkylation, and digestion of 10 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 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)

Contents: (10) S-Trap™ Micro Spin Columns

①	Solution 1	2x Lysis Solution	1 x 250 µL	10% (w/v) SDS, 100 mM TEAB in LC-MS grade Water, pH 7.55
②	Solution 2	Reductant	1 x 80.0 µL	120 mM TCEP in LC-MS grade Water
③	Solution 3	Alkylator	1 x 80.0 µL	500 mM MMTS in LC-MS grade IPA (mass shift is + 45.988, addition of SCH ₂)
④	Solution 4	Acidifier	1 x 100 µL	27.5% (v/v) Phosphoric Acid in LC-MS grade Water*
⑤	Solution 5	Binding/Wash Solution	1 x 900 µL (8.0 mL bottle)	100 mM TEAB (final) in 90% LC-MS grade MeOH, pH 7.5 Note: 8.1 mL LC-MS grade MeOH must be added to each bottle before use
⑥	Solution 6	Digestion Solution	1 x 1.0 mL	50 mM TEAB in LC-MS grade Water, pH 8.0
⑦	Solution 7	Elution Solution	1 x 1.0 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 **Denature Protein.** To 11.5 μ L of liquid sample, add 11.5 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 23.0 μ L of 1x Solution 1 to a solid sample. Sonicate or vortex to fully solubilize and denature proteins.
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 \times g for 8 minutes). Transfer clarified lysate to tubes. Pellet can be analyzed separately (see Appendix D).
- ② 4. **Reduce.** Add 1.0 μ L of **Solution 2 (Reducant)**. Vortex briefly and incubate at 55 °C for 15 minutes.
- ③ 5. **Alkylate.** Add 1.0 μ L of **Solution 3 (Alkylator)**. Vortex briefly and incubate at RT for 10 minutes.
- ⑤ 6. While the sample is incubating, add 8.1 mL of LC-MS grade MeOH to a bottle of **Solution 5 (Binding/Wash Solution)**. Mix thoroughly.
- ④ 7. **Acidify.** Add 2.5 μ L of **Solution 4 (Acidifier)** to the sample. Vortex briefly. pH paper can be used to ensure pH \leq 1. Proceed to the next step immediately.
- ⑤ 8. Add 165 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™ Micro Spin Column** in a 1.7 mL sample tube for waste flow-through. Transfer the entire sample, including any insoluble material, to the top of the column.

Note: The column reservoir can hold ~275 μ L of solution. For larger volumes, the column can be loaded multiple times with the solution from Step 8. When transferring into the column, do not disturb the matrix.
10. **Trap Protein.** Centrifuge the **S-Trap™ Micro Spin Column** and waste flow-through tube at 10,000 \times 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 \times 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 contain end in a Luer taper. A vacuum manifold or positive pressure can be used to draw solution through the column.
- ⑤ 11. **Clean Protein.** Add 150 μ L of **Solution 5 (Binding/Wash Solution) with MeOH added** to the **S-Trap™ Micro Spin Column** and centrifuge at 10,000 \times 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 the column makes it easier to track rotations.
12. Centrifuge the **S-Trap™ Micro Spin Column** at 10,000 \times g for 1 minute to fully remove all Binding/Wash Solution.
13. Transfer the protein-containing **S-Trap™ Micro Spin Column** to a clean 1.7 mL sample tube for digestion.
- ⑥ 14. Dilute trypsin in **Solution 6 (Digestion Solution)** to a final volume of 20.0 μ L, ensuring that the amount of trypsin 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).
15. Transfer the entire 20.0 μ L of trypsin 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 briefly using a benchtop centrifuge. If any solution flows through, pipette it back on top of the column.
16. **Incubate & Digest.** Loosely screw the cap on the **S-Trap™ Micro Spin Column** to limit evaporative loss so that the 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.
- ⑥ 17. **Elute 1.** Remove the **S-Trap™ Micro Spin Column** with sample tube from the incubator and add 40.0 μ L of **Solution 6 (Digestion Solution)** to the top of the column. Centrifuge at 10,000 \times g for 1 minute.
- ⑦ 18. **Elute 2.** Add 40.0 μ L of **Solution 7 (Elution Solution)** to the top of the **S-Trap™ Micro Spin Column**. Centrifuge at 10,000 \times g for 1 minute.
19. **Elute 3.** If hydrophobic peptides are of interest, add 40.0 μ L 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™ Micro Spin Column**. Centrifuge at 10,000 \times g for 1 minute.
20. Lyophilize or SpeedVac eluted peptides collected from Steps 17-19 in the sample tube. Resuspend as needed for subsequent analysis (e.g. Aqueous Buffer A such as 5% ACN, 0.1% FA).