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CATALOG NUMBER: P002-96MCR-0001/3/10PL

S-Trap™ 96-well Micro Plate

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. **S-Trap™ 96-well Micro Plates** allow for the solubilization, reduction, alkylation, and digestion of **≤ 100 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 - 100 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)*

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

Equipment/Materials (Not Included)

- pH meter with reference solutions
- Balance
- 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)
- 1.0 mL and 2.0 mL square well 96-well plates
- Silicone mat

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

Protein Sample	Your sample	1 µg – 100 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**	2x Lysis Solution	10% (w/v) SDS, 100 mM TEAB 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	27.5% (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.** 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).
4. **Reduce.** Add 1.0 µL of **Solution 2 (Reductant)**. Incubate at 55 °C for 15 minutes.
5. **Alkylate.** Add 1.0 µL of **Solution 3 (Alkylator)**. Incubate at RT for 10 minutes.
6. **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.
7. Add 165 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.*
8. Place an **S-Trap™ 96-well Micro Plate** atop a 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 7. When transferring into the well, do not disturb the matrix.*
9. **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.*
10. **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.*
11. Move the **S-Trap™ 96-well Micro Plate** atop a 1.0 mL 96-well plate for collection.
12. Dilute trypsin or trypsin/Lys-C 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 – 1:100 (w/w) ratio with the total amount of protein in the sample (e.g. 1 µg - 10 µg per 100 µg of sample).
13. Slowly and carefully transfer the entire 20.0 µL of trypsin or trypsin/Lys-C 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.
14. **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.*
15. **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.
16. **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.
17. **Elute 3.** If hydrophobic peptides are of interest, add 40.0 µL of **Solution 8 (Elution Solution 2)** to all wells of the **S-Trap™ 96-well Micro Plate**. Centrifuge at 2,000 x g for 2 minutes.
18. Lyophilize or SpeedVac eluted peptides collected from Steps 15-17 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).