CATALOG NUMBER: C002-MIDIX-0010/40/80PK (formerly C02-midi-10/20/40)

S-Trap™ Midi Spin Column

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™ Midi Spin Columns** allow for the solubilization, reduction, alkylation, and digestion of **≤ 1 mg 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 mg 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 channel pipettes (P2 through P1000) and corresponding tips
- 15.0 mL sample tubes
- Centrifuge with rotor for 15 mL falcon tubes capable of 4,000 x g
- 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)

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

Protein Sample	Your sample	≤ 1 mg
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	12.0% (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 250 µL of liquid sample, add 250 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 500 µL 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 x g for 8 minutes). Transfer clarified lysate to tubes. Pellet can be analyzed separately (see Appendix D).
4. **Reduce.** Add 20.0 µL of **Solution 2 (Reductant)**. Vortex briefly and incubate at 55 °C for 15 minutes.
5. **Alkylate.** Add 20.0 µL of **Solution 3 (Alkylator)**. Vortex briefly and incubate at RT for 10 minutes.
6. **Acidify.** Add 50.0 µL of **Solution 4 (Acidifier)** to the sample. Vortex briefly. pH paper can be used to ensure pH ≤ 1. Proceed to the next step immediately.
7. Add 3.3 mL 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™ Midi Spin Column** in a 15.0 mL sample tube for waste flow-through. Transfer the entire sample, including any insoluble material, to the top of the column.
   Note: *When transferring into the column, do not disturb the matrix.*
9. **Trap Protein.** Centrifuge the **S-Trap™ Midi Spin Column** and waste flow-through tube at 4,000 x 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 8,000 x g for 5 minutes. Discard flow-through and return the column to the 15.0 mL sample tube.
   Note: *A vacuum manifold or positive pressure can be used to draw solution through the column.*
10. **Clean Protein.** Add 3.0 mL of **Solution 5 (Binding/Wash Solution) with MeOH added** to the **S-Trap™ Midi Spin Column** and centrifuge at 4,000 x 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 15.0 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.*
11. Centrifuge the **S-Trap™ Midi Spin Column** at 4,000 x g for 1 minute to fully remove all Binding/Wash Solution.
12. Transfer the protein-containing **S-Trap™ Midi Spin Column** to a clean 15.0 mL sample tube for digestion.
13. Dilute trypsin or trypsin/Lys-C in **Solution 6 (Digestion Solution)** to a final volume of 350 µL, ensuring that the amount of trypsin 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).
14. Transfer the entire 350 µL of trypsin or trypsin/Lys-C solution to the **S-Trap™ Midi 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.*
15. **Incubate & Digest.** Loosely screw the cap on the **S-Trap™ Midi Spin Column** to limit evaporative loss so that 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 15.0 mL tube in a 47 °C water bath or humidified incubator for 1 - 2 hours. If using a water bath, make sure the column matrix sits below the water level to ensure even heating.
16. **Elute 1.** Remove the **S-Trap™ Midi Spin Column** with sample tube from the incubator and add 500 µL of **Solution 6 (Digestion Solution)** to the top of the column. Centrifuge at 4,000 x g for 1 minute.
17. **Elute 2.** Add 500 µL of **Solution 7 (Elution Solution)** to the top of the **S-Trap™ Midi Spin Column**. Centrifuge at 4,000 x g for 1 minute.
18. **Elute 3.** If hydrophobic peptides are of interest, add 500 µL **Solution 8 (Elution Solution 2)** to the top of the **S-Trap™ Midi Spin Column**. Centrifuge at 4,000 x g for 1 minute.
19. Lyophilize or SpeedVac eluted peptides collected from Steps 16-18 in the sample tube. Resuspend as needed for subsequent analysis (e.g. Aqueous Buffer A such as 5% ACN, 0.1% FA).