

Si-Trap™ 96-well Mini Kit



The ProtiFi™ **Si-Trap™** technology allows separation and capture of multiple classes of biomolecules from a single sample. The **Si-Trap™ 96-well Mini Kit** enables simultaneous analysis of lipids, metabolites, and proteins at a range of **25 - 200 µg of total protein**. Lipid Elution Solution is not included and needs to be purchased separately if analyzing lipids.

Additional Reagents/Solutions (Not Included)

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

- Sample (**25 µg - 200 µg of total protein**)*
- Protease of choice (e.g. Trypsin; 1 µg per 10 µg sample)**
- LC-MS grade Water
- LC-MS grade Acetonitrile (ACN)
- LC-MS grade Formic Acid (FA)
- Solution L (Lipid Elution Solution), sold separately (optional)

* **Sample cannot contain detergent**

** **Proteases are susceptible to freeze-thaw cycles**

Equipment/Materials (Not Included)

- 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 80 °C)
- Humidified incubator (adjusted to 47 °C)
- Lyophilizer or SpeedVac
- Positive pressure apparatus or vacuum manifold (optional)
- Sonicator (optional, recommended)
- Chemical Fume Hood

Contents: (1) Si-Trap™ 96-well Mini Plate, (1) 2.0 mL 96-well plate, (2) 1.0 mL 96-well plates, (2) silicone mats

①	Solution 1	1x Lysis Solution A	2 x 2.5 mL	Highly acidic solution to solubilize sample 
②	Solution 2	Binding Solution A	1 x 5.0 mL	Volatile buffer with alcohol to facilitate protein trapping
③	Solution 3	Metabolite Elution Solution	1 x 26.0 mL	MS compatible volatile buffer for metabolite elution
4A 4R 4B	Solution 4*	Reductant/Alkylator	1 x 5.0 mL, 1 x 500 µL, 1 x 300 µL	Reduces and prevents reformation of disulfide bonds
⑤	Solution 5	Wash Solution	3 x 26.0 mL	Volatile buffer with alcohol to facilitate protein cleaning
⑥	Solution 6	Digestion Solution	1 x 30.0 mL	MS compatible volatile buffer for optimal digestion
⑦	Solution 7	Elution Solution	1 x 8.0 mL	MS compatible volatile buffer for optimal elution

All provided solutions, besides Solution 1, can be stored up to 1 year at -20 °C. After thawing at 4 °C, mix thoroughly to ensure any precipitate is dissolved. Solution 1, closed tightly, can be stored at RT up to 1 year; do not heat. The Si-Trap™ 96-well Mini Plate can be stored up to 1 year at 4 °C.

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

*Prior to starting:



If you intend to use the full kit, add 250 µL of **Solution 4A (Alkylator)** and 417 µL **Solution 4R (Reductant)** to the bottle of **Solution 4B (Reductant/Alkylator Buffer)**. If you will use less than the full kit, per sample add 2.6 µL of **Solution 4A (Alkylator)** and 4.3 µL **Solution 4R (Reductant)** to 52.0 µL of **Solution 4B (Reductant/Alkylator Buffer)**. Mix thoroughly.

Protocol:

- ① **1. CAUTION CAUSTIC SOLUTION; USE PROPER PROTECTIVE EQUIPMENT: Lyse.** To 23.0 µL of liquid sample, add 23.0 µL of **Solution 1 (1x Lysis Solution A)**. For solid samples, add 46.0 µL of **Solution 1 (1x Lysis Solution A)**. Sonicate or vortex to fully solubilize. Add **Binding Solution A** as soon as the sample is dissolved. We **recommend** performing this step in a chemical fume hood.
Note: Sample cannot contain detergent. Solution 1 (1x Lysis Solution A) is compatible with BCA at 1:100 dilution.
2. If sample is viscous due to DNA presence, shear thoroughly by probe sonication. Unsheared DNA may clog the matrix.
- ② **3. Add 46.0 µL of Solution 2 (Binding Solution A) to the sample. Mix thoroughly. Do NOT centrifuge.**
- ③ **4. Collect Lipids (optional).** If lipids are of interest, add 184 µL of **Solution L (Lipid Elution Solution)**, sold separately, to the sample; after lysis. Ensure thorough mixing of the liquids. We **recommend** performing this step in a chemical fume hood.
5. Place the **Si-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 **Si-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 µL of solution. For larger volumes, the well can be loaded multiple times with the solution from Step 4. When transferring into the well, do not disturb the matrix.
6. **Trap.** Centrifuge the **Si-Trap™ 96-well Mini Plate** and collection plate at 2,000 x g for 2 minutes. Visually confirm that all solution passed through. Keep flow-through. Place the **Si-Trap™ 96-well Mini Plate** atop a 1.0 mL 96-well plate.
Note: A vacuum manifold or positive pressure can be used; ensure that all wells empty.
- ③ **7. Elute Metabolites.** Add 250 µL of **Solution 3 (Metabolite Elution Solution)** to the **Si-Trap™ 96-well Mini Plate** and centrifuge at 2,000 x g for 2 minutes. Visually confirm that all solution passed through the well. If not, centrifuge again for until all solution has passed through the plate.
8. **Collect Lipids (optional).** Transfer flow-through from Steps 6 and 7 into an appropriately sized tube. Mix thoroughly then settle to allow full phase separation to occur. Carefully pipette off the top layer containing lipids into a new appropriately sized container. SpeedVac collected lipids. Resuspend or store as needed for analysis.
9. **Collect Metabolites.** The bottom layer contains metabolites. Lyophilize or SpeedVac eluted metabolites collected in the same 1.0 mL 96-well plate. Resuspend or store as needed for subsequent analysis. Place the **Si-Trap™ 96-well Mini Plate** atop the 2.0 mL 96-well plate.
- ④ **10. Reduce and Alkylate.** Thoroughly vortex **Solution 4B (Reductant/Alkylator Buffer)** created prior to use. Add 50 µL of **Solution 4B (Reductant/Alkylator Buffer)** to the **Si-Trap™ 96-well Mini Plate**. Incubate at 80 °C for 30 minutes.
- ⑤ **11. Clean Proteins.** Add 250 µL of **Solution 5 (Wash Solution)** to the **Si-Trap™ 96-well Mini Plate** and centrifuge at 2,000 x g for 2 minutes. Visually confirm that all solution passed through the column. Repeat washes 2 more times (3 total); 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 **Si-Trap™ 96-well Mini Plate** atop a 1.0 mL 96-well plate for collection.
- ⑥ **13. Dilute trypsin in Solution 6 (Digestion Solution) to a final volume of 125 µL, 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 µg per 100 µg of sample).**
14. Transfer the entire 125 µL of trypsin solution to the **Si-Trap™ 96-well Mini Plate**, ensuring the matrix is fully covered by digestion solution.
Note: The matrix is hydrophilic and will absorb the solution; no centrifugation is necessary.
- ⑥ **15. Incubate and Digest.** Loosely cover the **Si-Trap™ 96-well Mini Plate** to limit evaporative loss without making an air-tight seal. Place plate atop another 1.0 mL 96-well plate in a 47°C humidified incubator for 1-2 hours. If desired, after 1 hour, add 80.0 µL of **Solution 6 (Digestion Solution)** to all wells. Loosely cover; place back in a 47 °C humidified incubator for 1 hr.
Note: Some dripping may occur during incubation; this is not of concern. Do NOT shake.
- ⑥ **16. Elute 1.** Add 80.0 µL of **Solution 6 (Digestion Solution)** to all wells of the **Si-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 µL of **Solution 7 (Elution Solution)** to all wells of the **Si-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 µL 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 **Si-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).