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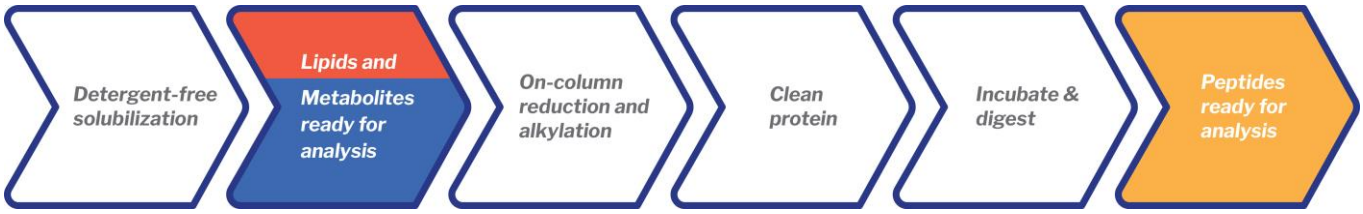
Si-Trap™ 96-well Mini Kit



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-TrapTM 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:**

4A 4R 4B 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, sheer 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.

L **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.

4B **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).