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🌐 S-Trap™ column digestion protocol (Protifi) of proteins for LC-MS / proteomics

📁 In 1 collection

DOI

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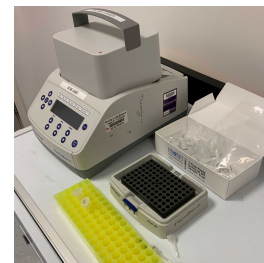
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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol details the in-house BioMS procedure of S-Trap™ protein clean-up and subsequent column digestion/conversion of protein to peptide using trypsin.

It is adapted from the long protocol from Protifi (as on August 2022) - <https://files.protifi.com/protocols/s-trap-micro-long-4-7.pdf>

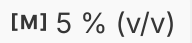
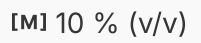
Attachments



[iiabptmp.docx](#)

173KB

Guidelines

- You may purchase S-trap columns and other consumables directly from us at the BioMS core facility on PPMS - <https://corefacilities.manchester.ac.uk/?BioMS>).
- Allow  02:30:00 for this process
- This protocol is to be used for protein loads columns are best for loadings less than  100 µg of starting material (protein).¹
- You have cell or tissue lysates in  25 µL or  50 µL of S-Trap lysis buffer (5% SDS with 50 mM TEAB pH 7.5). If your lysate volume is lower than these two volumes, make up to either with 5% S-Trap lysis buffer (working concentration).²
- SDS is essential for the S-trapping process and subsequent clean-up, it is important that samples contain close to  5 % (v/v) SDS. This can be achieved by dilution of the sample using the  10 % (v/v) stock of S-trap lysis buffer.
- Protein lysates have been sheared of nucleic acid (e.g. from BioMS's Covaris LE220+ AFA sonication system). Alternatively, a nuclease such as Benzonase may be used.³
- Protein lysates have been **reduced** and **alkylated**⁴ and clarified by centrifuging at  14000 x g for  00:10:00 (see reduction and alkylation protocol in this series).
- The protein concentration of a pool of the protein lysates has been quantified and samples prepared to be less than  4 µg/µL of protein (Use the Millipore Direct Detect with the 5% SDS calibration file to check).
- Please take care not to touch the S-trap matrix in the column with pipette tips during the process.

Notes:

1. If you have more than  100 µg of protein - use the S-trap plate method "S-Trap™ 96-well plate digestion protocol" in this collection.
2. Alternatively, if your lysate volume is larger than 50uL, simply remove a  25 µL sample aliquot for processing.
3. This step is essential as omitting it may clog the S-Trap column. If you do not have access to ultrasonication, see protocol from [EMDmillipore](#) for details.
4. If it is thought that the insoluble pellet contains proteins of interest, then proceed with homogenising (with a pipette and tip) the insoluble pellet as much as possible with fresh 5% S-trap lysis buffer. A suspension of



insoluble particles may be formed this way. Proceed with a separate S-trap column to process this additional sample.

Materials

Locate the following buffers and reagents:

A	B
Location	Buffer/reagent
Bench (orange tray)	-- 12% phosphoric acid -- S-Trap binding buffer (90% aqueous methanol containing a final concentration of 100 mM TEAB, pH 7.1) -- 0.1% formic acid in water -- 0.1% formic acid in 30% acetonitrile -- MTBE / Methanol solution (10/3 (v/v))
Freezer 3	Aliquots of trypsin (10 uL at 2ug.uL ⁻¹)

Locate the following consumables:

S-Trap columns for processing (CO₂-micro) - please purchase from in advance from BioMS core facility on PPMS - <https://corefacilities.manchester.ac.uk/?BioMS>).

1.5 mL (i), and 2 mL (ii) Eppendorf tubes

for

(i) diluting samples (if necessary), and for digestion step.

and

(ii) collecting unbound material from the S-trapping process.

Identify the following equipment that you will use:

- One each of 20 µL, 200 µL, and 1000 µL pipettes.
- Eppendorf centrifuge 5430R (or equivalent benchtop centrifuge that will centrifuge 2 mL Eppendorf tubes at 14000 x g).
- Depending on the number of protein samples you need to process, you may find that you need additional S-Trap binding buffer, there are aliquots of 5 mL of 100 millimolar (mM) TEAB at pH 7.1 stored in 50 mL Falcon tubes in freezer 3. - take one, thaw at Room temperature, and add 45 mL of methanol (locate in fume hood) to make a final volume of 50 mL to use.
- A vortex mixer (if diluting samples)
- An Eppendorf Thermomixer with 1.5 mL thermoblock and set it to 47 °C, 01:00:00, and a speed of 0 rpm (i.e. no shaking).

Catalogue numbers:

ortho-Phosphoric acid 85% Merck MilliporeSigma (Sigma-Aldrich) Catalog #1005732500

Methanol Optima™ LC/MS Grade Fisher Chemical Fisher Scientific Catalog #A456-4

✂ Pierce™ 0.1% Formic Acid (v/v) in Acetonitrile, LC-MS Grade **Thermo Fisher Catalog #85174**

✂ Pierce™ 0.1% Formic Acid (v/v) in Water, LC-MS Grade **Thermo Fisher Catalog #85170**

✂ tert-Butyl methyl ether 99% **Alfa Aesar Catalog #L14030**

✂ Trypsin TPCK Treated **Worthington Biochemical Corporation Catalog #LS003740**

✂ SDS **Merck MilliporeSigma (Sigma-Aldrich) Catalog #75746**

✂ Triethylammonium bicarbonate (TEAB) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T7408**

✂ S-Trap™ micro columns ($\leq 100 \mu\text{g}$) **Protifi Catalog #C02-micro** (may be purchased in advance from BioMS core facility on PPMS - <https://corefacilities.manchester.ac.uk/?BioMS>).

Safety warnings

- ⚠ Please refer to the copies of Risk Assessment Forms held in both B2071 and B2075 for hazards to health, and other identified hazards and risks, associated with the use of this protocol before starting.

Before start

Locate the Eppendorf Thermomixer

Use the  1.5 mL adaptor for the Eppendorf Thermomixer, and set the thermomixer to  47 °C ,

 01:00:00 , and a speed of  0 rpm **(i.e. no shaking)**.

Sample preparation

10m

- 1 To the reduced and alkylated sample of volume either of $\text{25 } \mu\text{L}$ or $\text{50 } \mu\text{L}$, add a volume of $\text{2.5 } \mu\text{L}$ or $\text{5.0 } \mu\text{L}$ respectively of $\text{12 \% (v/v)}$ aqueous phosphoric acid at a ratio of 1:10 for a final concentration of $\text{1.2 \% (v/v)}$ phosphoric acid and vortex mix.



Note

Total volume is now either $\text{27.5 } \mu\text{L}$ or $\text{55 } \mu\text{L}$.

Note

This step is essential to completely denature proteins and trap them efficiently.

The pH will be $\leq \text{pH } 1.0$. If the sample pH is not $\leq \text{pH } 1.0$, add additional phosphoric acid to reach $\text{pH } \leq \text{pH } 1.0$.

A quick way to check the pH is to spot $\text{2 } \mu\text{L}$ of the acidified lysate on a strip of filter paper.

The final phosphoric acid concentration is different between S-Trap micros, and minis/midis.

- 2 Add $\text{165 } \mu\text{L}$ or $\text{330 } \mu\text{L}$ of S-Trap binding buffer to the $\text{27.5 } \mu\text{L}$ or $\text{55 } \mu\text{L}$ volumes of acidified protein lysates respectively and mix.



Note

Total volume is now $\text{192.5 } \mu\text{L}$ for the $\text{25 } \mu\text{L}$ starting volume, or $\text{385 } \mu\text{L}$ for the $\text{50 } \mu\text{L}$ starting volumes.

Sample Trapping

30m



3 Place the S-Trap column on top of a  2 mL Eppendorf tube. This will collect the flow-through.

4 Add enough of the acidified methanolic lysate to the S-Trap column. 

Note

Do not add more sample than will fit the narrow “stem” of the S-Trap column. If the volume to be loaded is larger than will fit in the stem, then proceed to centrifugation (next step).

5 Centrifuge the column/tube combination at  4000 rcf for  00:02:00 in the Eppendorf 5430R centrifuge. 

Note

Protein should be trapped within the protein-trapping matrix of the column. It is important not to let the liquid that passes through the S-Trap to come in contact with the protein-trapping matrix of the column.

6 Repeat the previous two steps if there is additional sample to be processed.

Note

Visually confirm all sample has passed through the column; if not, centrifuge again until all sample has passed through.

Sample Washing

30m

7 Wash captured protein with **one** wash of  150 μ L of MTBE solution, simply add  150 μ L of the MTBE solution to the column, and spin at  4000 x g for  00:02:00. This will remove methanol-insoluble biomolecules from the quartz filter.   

2m

Note

For best results, rotate the S-Trap micro units (like a screw or knob) 180 degrees between the centrifugations of binding and wash steps. This is especially important when using a fixed-angle rotor because the spin column does not experience homogenous flow. A mark on the outside edge with a "Sharpie" type marker during centrifugation makes it easy to track rotations.

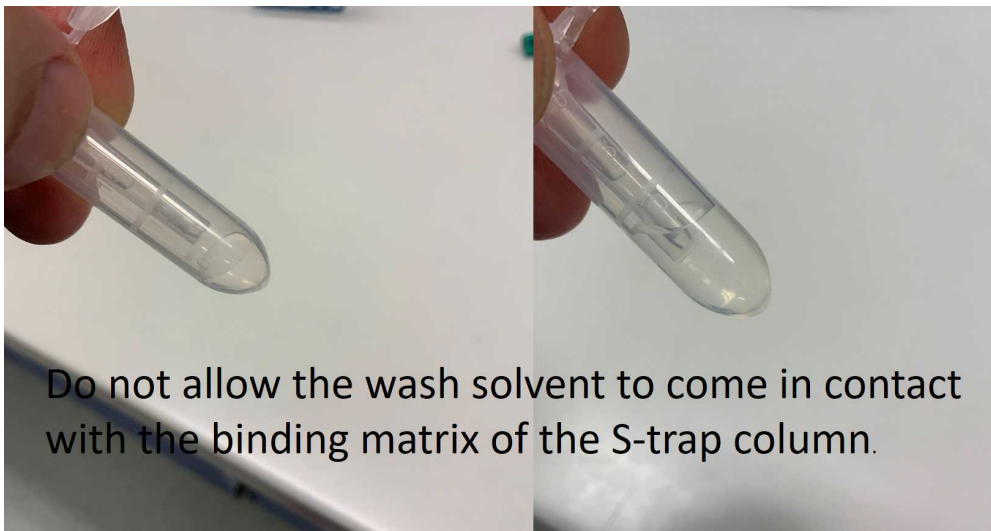
- 8 Following this, perform **four** washes of  150 μL of S-Trap binding buffer, again, add  150 μL of the S-trap binding buffer, and centrifuge at  4000 x g for  00:02:00 .

2m



Note

Note: If you wish, you may transfer the flow through and washes back into an Eppendorf sample tube after each centrifugation step, otherwise empty the collection tube so that the washes do not come in contact with the binding matrix of the S-trap column. If discarding the washes then collect in a beaker and dispose in the acetonitrile/solvent waste when finished.



Do not allow the wash solvent to come in contact with the binding matrix of the S-trap column.



Note

- Note: Depending on the number of protein samples you need to process, you may find that you need additional S-Trap binding buffer.
- If so, there are aliquots of 5 mL of 100 millimolar (mM) TEAB at 7.1 stored in 50 mL Falcon tubes in freezer 3.
- - take one out, thaw at Room temperature , and add 45 mL of methanol (located in fume hood) to make a final volume of 50 mL , mix, transfer to the bin.

Digestion

1h

9 Move S-Trap column to a clean digestion 1.5 mL Eppendorf tube.

10 Locate the trypsin aliquots. They are in the top shelf of freezer 3.

Note

Trypsin must be added to the protein at a ratio of 1:10 wt:wt (enzyme:protein).

11 The frozen aliquots are at a volume of 10 μ L containing 20 μ g of trypsin (concentration of 2 μ g/ μ L).

20 μ L of digestion buffer is needed for each column.

If working from 50 μ g of protein starting material, then add 75 μ L of digestion buffer to the trypsin aliquot. This gives a total volume of 85 μ L , enough for 4 S-Trap digestions.

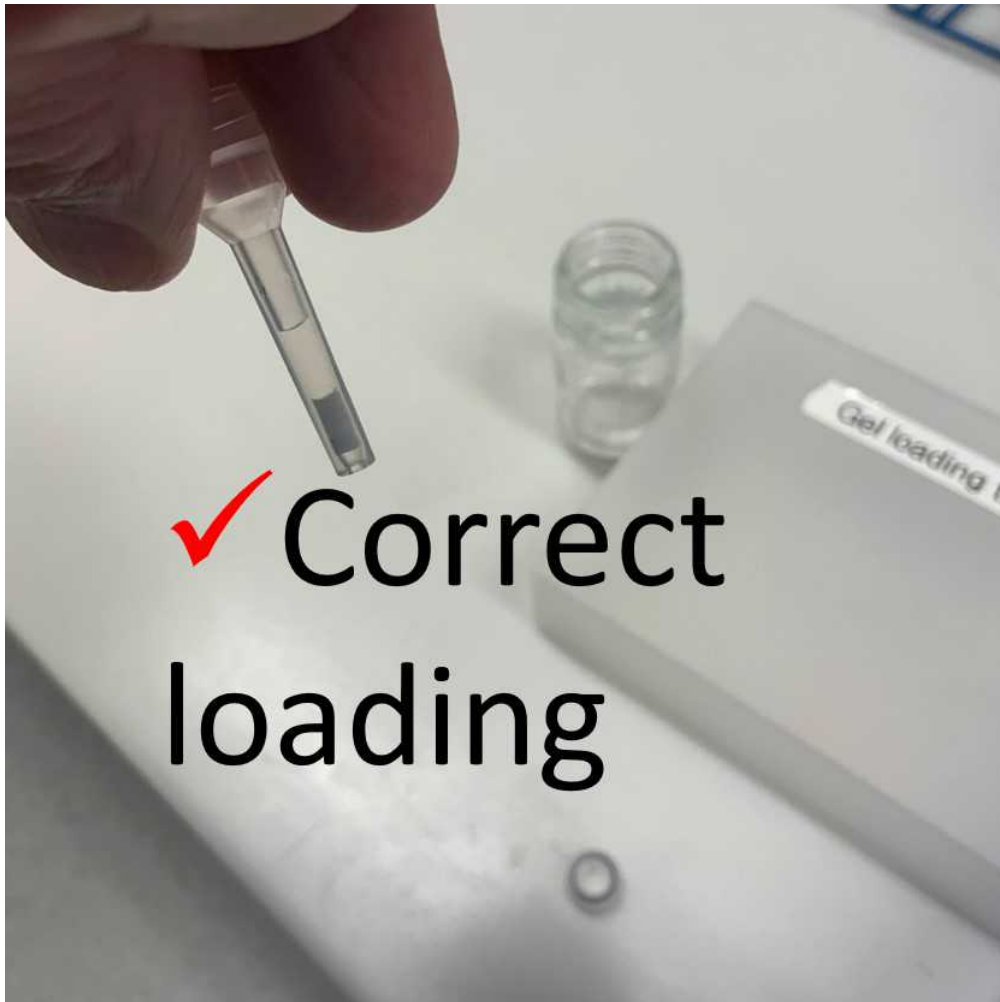
Note

If your amount of protein starting material is 25 μ g , make up the aliquot with 155 μ L of digestion buffer, enough for 8 S-Trap column digestions.

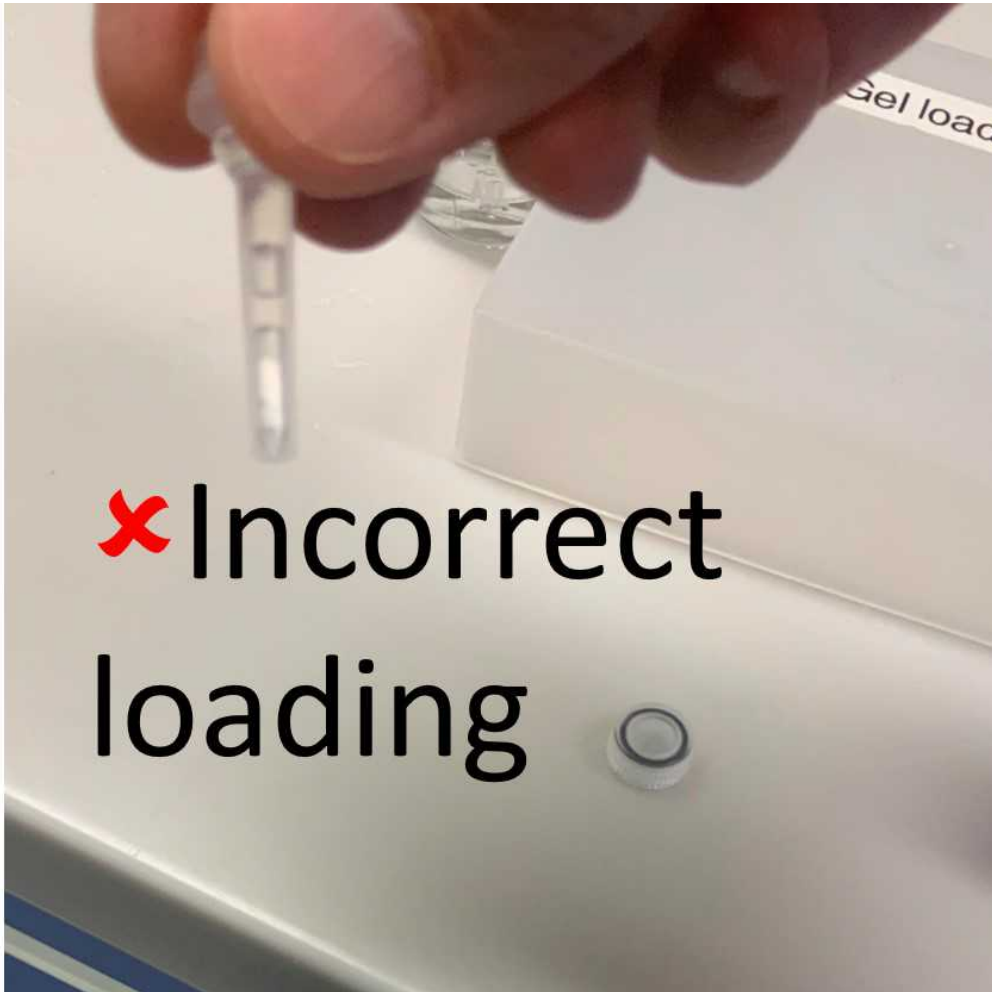
12 Add 20 μ L of digestion buffer containing protease at 1:10 wt:wt into the top of the micro column. Use a gel loading tip (blue box) for this step.

Note

IMPORTANT! – Ensure there is no air bubble between the digestion buffer and the column matrix. The S-Trap binding matrix is hydrophilic and will absorb the digestion buffer when incubating.



Correct loading of digestion buffer (no air bubble)



incorrect loading of digestion buffer (air bubble present in column)

Bubbles prevent the digestion buffer from entering the s-trap column matrix.

- 13 Cap the S-Trap column loosely to limit evaporative loss. A suggested way to do this is to close the cap until you feel resistance, then loosen one half-turn.
- 14 Incubate in the Eppendorf thermomixer for 01:00:00 at 47 °C for trypsin.

Note

Some dripping may occur during incubation; this is not of concern. REMEMBER - DO NOT SHAKE.



1h



- 15 If you wish, you may also set up this digestion step overnight, with no impact on the S-trap process. To do this, set the Thermomixer to $37\text{ }^{\circ}\text{C}$ and incubate overnight, again with no shaking.



Elution of peptides

20m

- 16 Add $65\text{ }\mu\text{L}$ of digestion buffer to the S-Trap column. Centrifuge the column / tube at 4000 x g for $00:02:00$, and collect.

2m



- 17 Add $65\text{ }\mu\text{L}$ of 0.1% aqueous formic acid (FA) to the S-Trap column.



- 17.1 Centrifuge the column / tube at 4000 x g for $00:02:00$, and collect, this is now combined with the first elution through the centrifugation process.

2m



- 18 Add $30\text{ }\mu\text{L}$ of 30% aqueous acetonitrile containing 0.1% formic acid. Centrifuge the column / tube at 4000 x g for $00:02:00$, and collect.

2m



- 19 This elution assists in recovery of hydrophobic peptides. This is now combined with the the first and second elutions. The total volume will be approximately $200\text{ }\mu\text{L}$.

Note

The final acetonitrile concentration will be around $5\text{ }\%$ (v/v).

- 20 Proceed to R3 desalting or store in a refrigerator.



Note



When you are ready, please dispose of any solvent waste in the non-chlorinated waste drum.