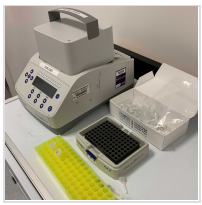

Sep 06, 2022

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

In 1 collection

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dx.doi.org/10.17504/protocols.io.yxmvmn6q9g3p/v1

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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

iiaebptmp.docx

DOI

dx.doi.org/10.17504/protocols.io.yxmvmn6q9g3p/v1

PROTOCOL CITATION

ronan.ocualain, Jamesallsey, Davidknight, Staceywarwood, Emmakeevill 2022. S-Trap™ column digestion protocol (Protifi) of proteins for LC-MS / proteomics. **protocols.io** https://protocols.io/view/s-trap-column-digestion-protocol-protifi-of-protei-cdtys6pw

COLLECTIONS

Researcher led sample preparation for LC-MS using the BioMS research core facility

KEYWORDS

Digestion protocol, S-Trap column, Eppendorf thermomixer, Mass spec analysis, proteomics, quartz, s-trapping, S-trap, Protifi, digestion, desalting, clean-up, LC-MS, off-line, offline, enzymatic digestion

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OWNERSHIP HISTORY

Jul 20, 2022 madhavi.d

Aug 15, 2022 ronan.ocualain

PROTOCOL INTEGER ID

67160

PARENT PROTOCOLS

Part of collection

Researcher led sample preparation for LC-MS using the BioMS research core facility

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+

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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 TEXT

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 (C02-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

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(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 **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% **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 **Sigma Catalog #75746**

Triethylammonium bicarbonate (TEAB) **Sigma Aldrich Catalog #T7408**

S-Trap™ micro columns (≤ 100 µg) **Protifi Catalog #C02-micro** (may be purchased in advance from BioMS core facility on PPMS - https://corefacilities.manchester.ac.uk/?BioMS).

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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 STARTING

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 **25 µL** or **50 µL** , add a volume of **2.5 µL** or **5.0 µL** respectively of **12 % (v/v)** aqueous phosphoric acid at a ratio of 1:10 for a final concentration of **1.2 % (v/v)** phosphoric acid and vortex mix.

Total volume is now either **27.5 µL** or **55 µL** .

This step is essential to completely denature proteins and trap them efficiently.
The pH will be ≤ **1.0** . If the sample pH is not ≤ **1.0** , add additional phosphoric acid to reach pH ≤ **1.0** .
A quick way to check the pH is to spot **2 µL** of the acidified lysate on a strip of filter paper.
The final phosphoric acid concentration is different between S-Trap micros, and minis/midis.

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2

Add **165 µL** or **330 µL** of S-Trap binding buffer to the **27.5 µL** or **55 µL** volumes of acidified protein lysates respectively and mix.

Total volume is now **192.5 µL** for the **25 µL** starting volume, or **385 µL** for the **50 µL** 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.

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  2m

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

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.

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

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Sample Washing 30m

7 2m

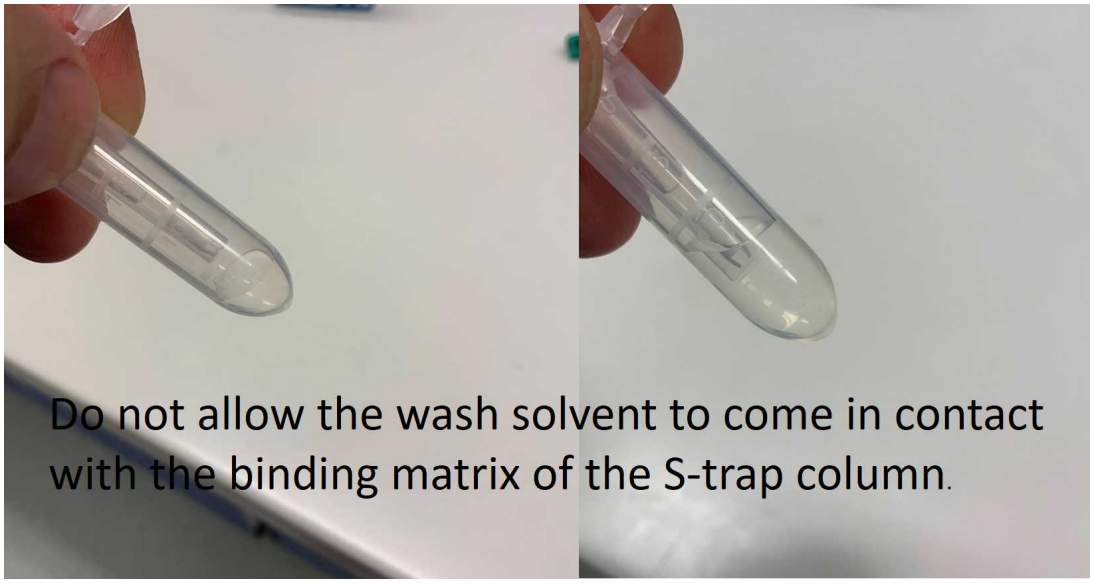
Wash captured protein with **one** wash of **150 µL** of MTBE solution, simply add **150 µL** of the MTBE solution to the column, and spin at **4000 x g** for **00:02:00** . This will remove methanol-insoluble biomolecules from the quartz filter.

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 2m

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** .

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.



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- 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.

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 µL** containing **20 µg** of trypsin (concentration of **2 µg/µL**).

20 µL of digestion buffer is needed for each column.

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

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

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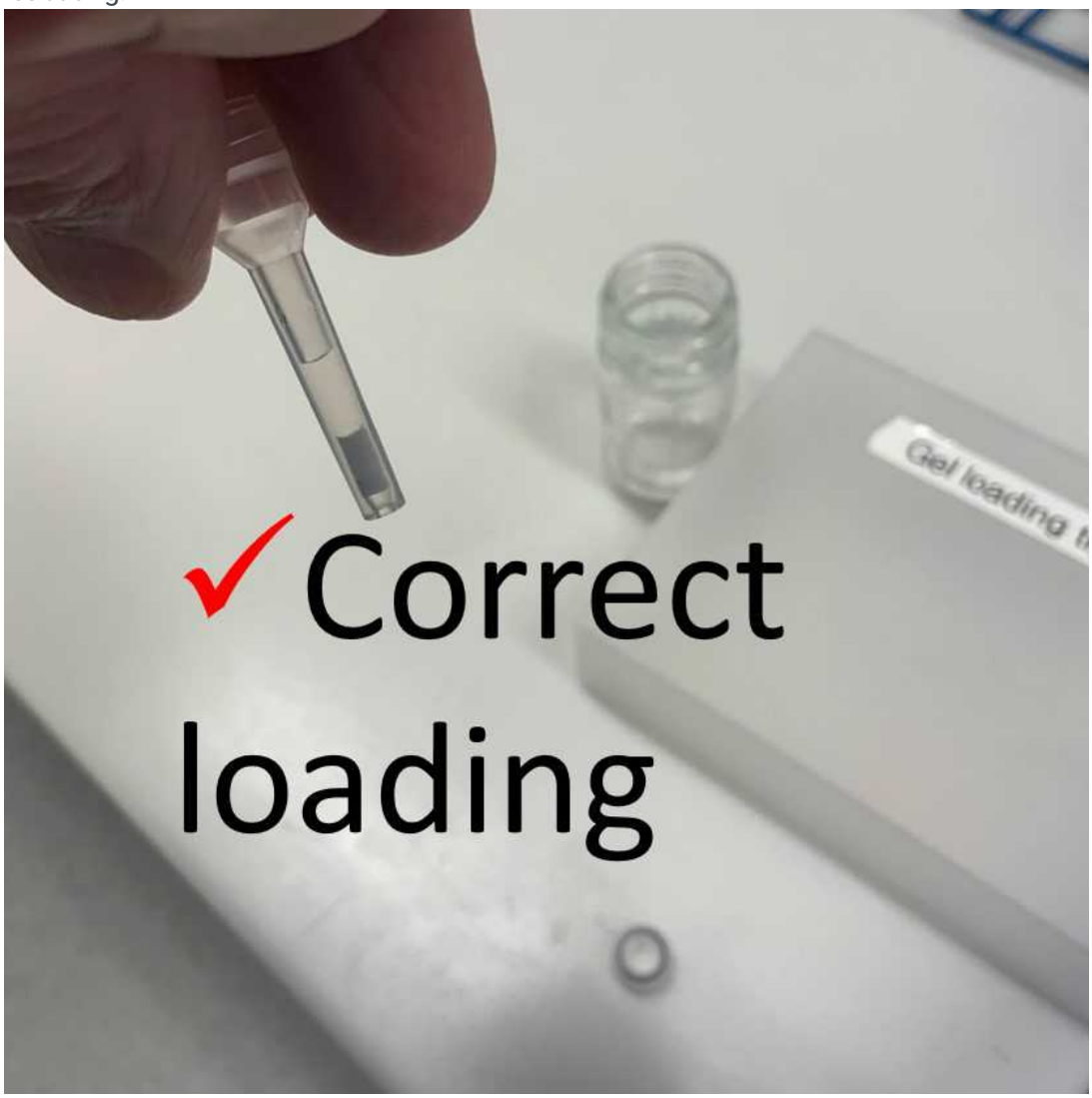
12

Add **20 µL** 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.

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)

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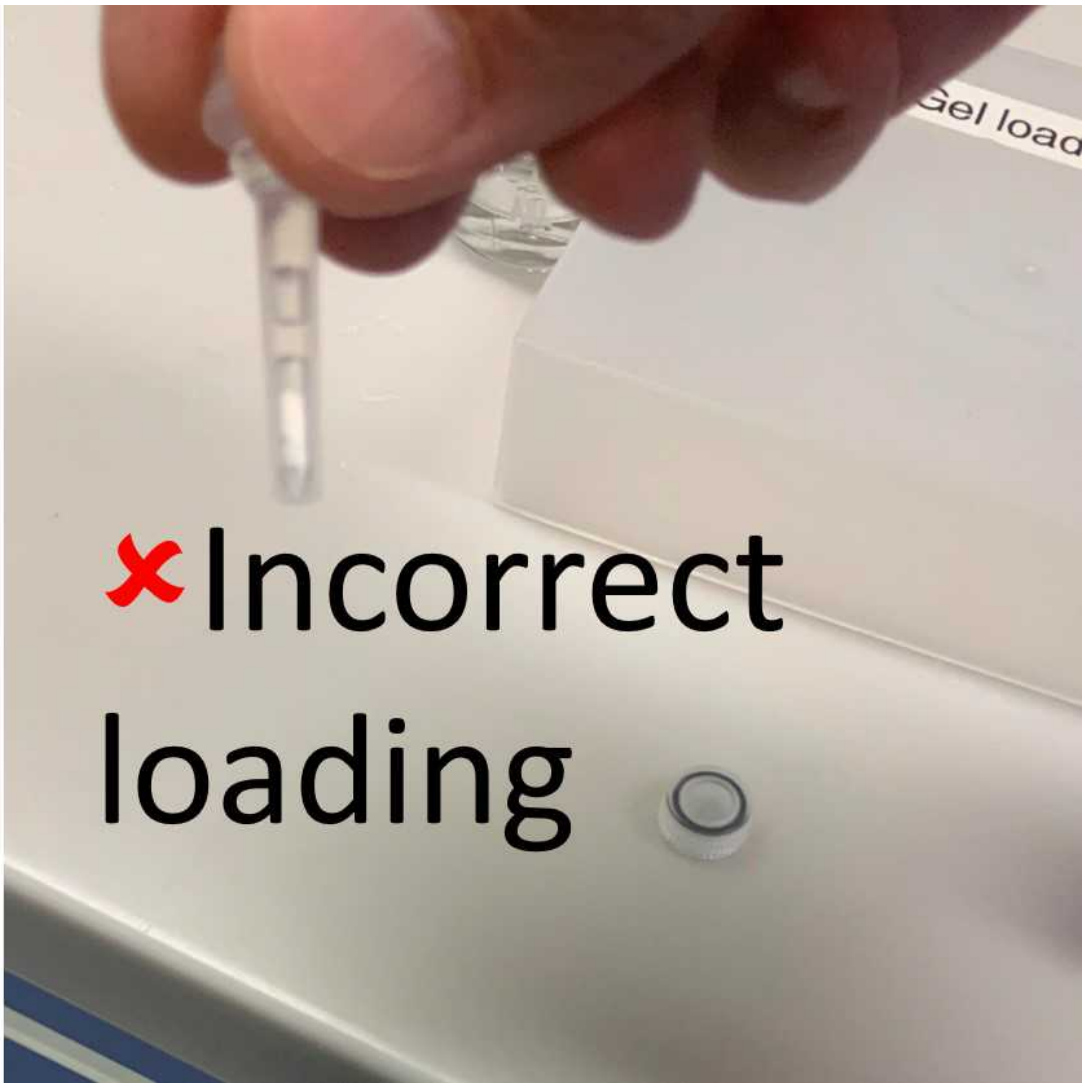

incorrect loading of digestion buffer (air bubble present in column)

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 1h

Incubate in the Eppendorf thermomixer for **01:00:00** at **47 °C** for trypsin.

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

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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 °C** and incubate overnight, again with no shaking.

Elution of peptides 20m

16 2m

Add **65 µL** of digestion buffer to the S-Trap column. Centrifuge the column / tube at **4000 x g** for **00:02:00** , and collect.

17

Add **65 µL** of 0.1% aqueous formic acid (FA) to the S-Trap column.

17.1 2m

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.

18 2m

Add **30 µL** of 30% aqueous acetonitrile containing 0.1% formic acid. Centrifuge the column / tube at **4000 x g** for **00:02:00** , and collect.

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 µL** .

> The final acetonitrile concentration will be around **5 % (v/v)** .

20

Proceed to R3 desalting or store in a refrigerator.

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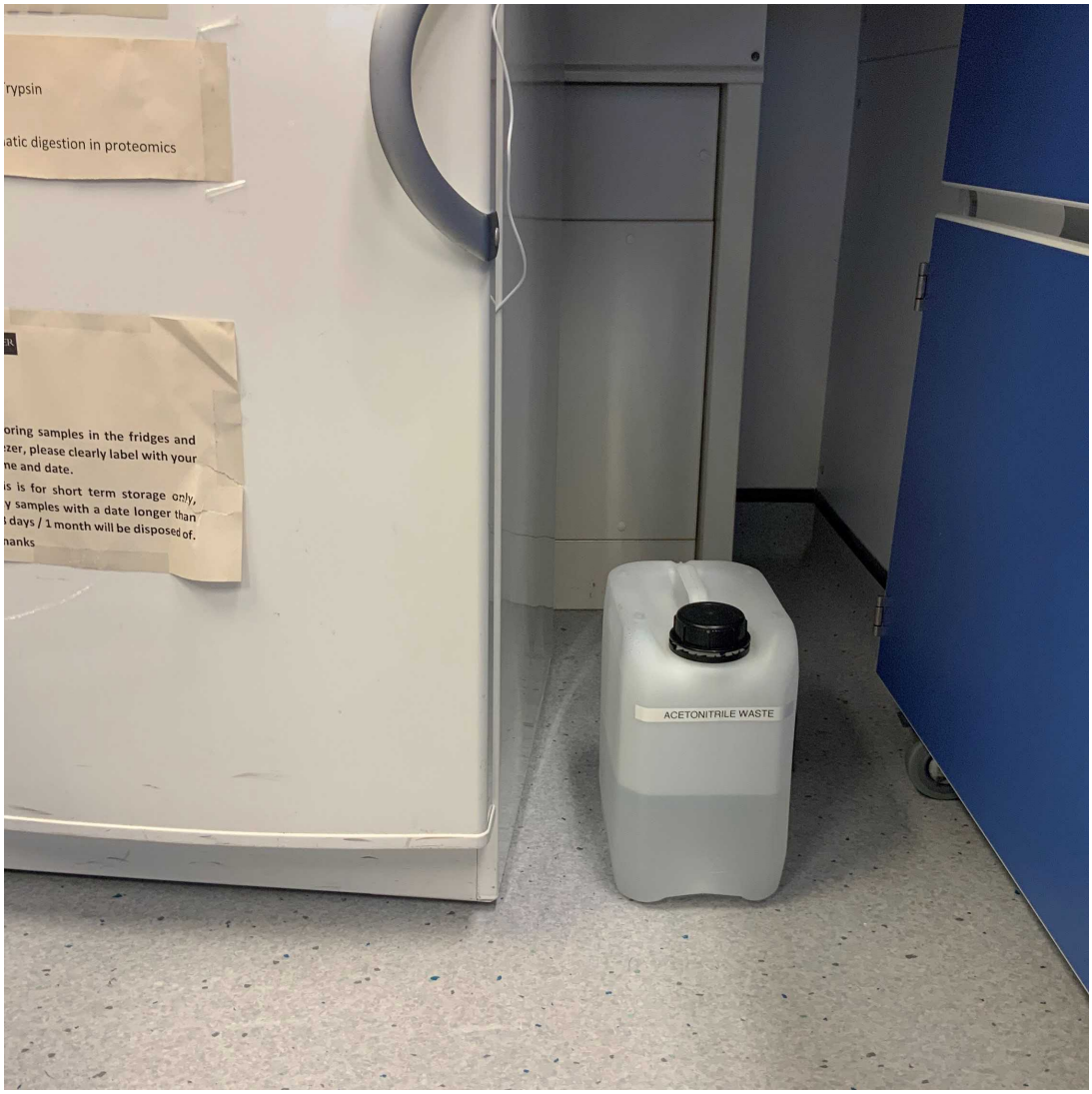

When you are ready, please dispose of any solvent waste in the non-chlorinated waste drum.

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