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

ronan.ocualain¹¹University of Manchester

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dx.doi.org/10.17504/protocols.io.261genmkgd47/v1

BioMS CRF, UoM



ronan.ocualain

ABSTRACT

This is a collection of protocols that covers the processing of most biological samples for proteomics, from collection of sample, up to data acquisition using LC-MS

ATTACHMENTS

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COLLECTION CITATION


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KEYWORDS

S-TrapTM column, Sample lysis, Speed vac, sample preparation, lc-ms, proteomics, biochemistry, reduction and alkylation, ultrasonication, micro-proteomics, plate-based, throughput, S-trap plate, protifi, covaris, millipore, direct detect, protein, peptides, mass spectrometry, desalting, cleanup, clean-up, processing, enzymatic digestion, trypsin, R3, reverse-phase

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ronan.ocualain

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GUIDELINES

This method is suitable for the preparation of a wide variety of sample types for LC-MS analysis. It is assumed that you have already had a discussion about your work before attempting the steps described here. If not, contact us at **bioms@manchester.ac.uk** for a chat.

BEFORE START

This method is suitable for the preparation of a wide variety of sample types for LC-MS analysis. It is assumed that you have already had a discussion about your work before attempting the steps described here. If not, contact us at **bioms@manchester.ac.uk**.

To get you started, we will provide you with Covaris lysis tubes for each sample that you wish to process, at cost.

MATERIALS TEXT

See individual protocols for the materials and consumables used.

To note, for the successful processing of microgram quantities of starting material, we rely on Covaris ultrasonication.

This is achieved by lysis and protein extraction taking place in small volumes of lysis buffer, with minimal processing steps. This minimises the chances of sample loss and subsequent sample recovery.

Other lysis and extraction methods can be used prior to proceeding to the S-trapping process.

SAFETY WARNINGS

- This protocol uses a number of chemicals and a risk assessment should be performed before starting.
- We have COSHH forms and risk assessments in a red folder in both B2071 and B2075 labs (above LDA1), where you can check the single substance risk assessment forms for each of the chemicals used in this protocol.
- None of the chemicals used in this workflow are CMRs (carcinogen, mutagens, or reproductive toxins).
- It is recommended that PPE be worn at all stages of the workflow, and remove gloves when using laptop keyboards for the Millipore Direct Detect and Covaris LE220+.

Above all, if you have any questions about H&S, or feel unsafe about any of the procedures

– PLEASE ASK and we will do our best to answer.



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This is a collection of protocols that covers the processing of most biological samples for proteomics, from collection of sample, up to data acquisition using LC-MS



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

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

Biological sample lysis and extraction using the Covaris LE220+ for LC-MS
Version 1
by **ronan.ocualain**
- 



Reduction and alkylation of protein lysates for LC-MS (proteomics) using dithiothreitol (DTT) and iodoacetamide (IAM)
Version 1
by **ronan.ocualain**
- 

Measuring protein concentration using the Merck Millipore Direct Detect Spectrometer
Version 1
by **ronan.ocualain**
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S-Trap™ plate digestion protocol (Protifi) of proteins for LC-MS / proteomics
Version 1
by **ronan.ocualain**
- 

S-Trap™ column digestion protocol (Protifi) of proteins for LC-MS / proteomics
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96-well plate R3 desalt and clean up protocol for mass spec analysis
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Using the Thermo SPD1010 speedvac concentrator centrifuge for drying down peptides for LC-MS analysis
Version 1
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Sample submission for LC-MS BioMS core facility at the University of Manchester
Version 1
by **ronan.ocualain**