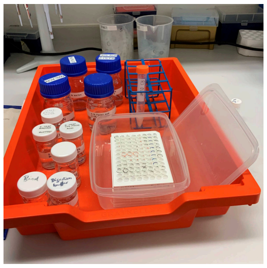
Sep 09, 2022

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



In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.261genmkdg47/v1

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The University of Manchester

DOI: **https://dx.doi.org/10.17504/protocols.io.261genmkdg47/v1**

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Protocol status: Working
We use this collection and it's working

Created: July 21, 2022

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Collection Integer ID: 67206

Keywords: S-Trap™ 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, sample preparation for lc, proteomic, bioms research core facility, sample preparation, processing of most biological sample, most biological sample, using lc, sample, data acquisition, collection of sample, preparation, lc

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

DOCX

iiaebptmp.docx
173KB

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.

Materials

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.

Troubleshooting

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.

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.

Attachments

DOCX

iiaebptmp.docx

173KB

Files

SEARCH

Protocol

NAME

Biological sample lysis and extraction using the Covaris LE220+ for LC-MS

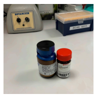
VERSION 1

CREATED BY

ronan o'cualain
Inoviv

OPEN →

Protocol



NAME

Reduction and alkylation of protein lysates for LC-MS (proteomics) using dithiothreitol (DTT) and iodoacetamide (IAM)

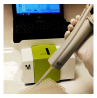
VERSION 1

CREATED BY

ronan o'cualain
Inoviv

OPEN →

Protocol



NAME

Measuring protein concentration using the Merck Millipore Direct Detect Spectrometer

VERSION 1

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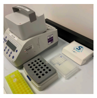
Protocol



NAME

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

VERSION 1

CREATED BY

ronan o'cualain
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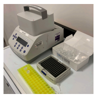
Protocol



NAME

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

VERSION 1

CREATED BY

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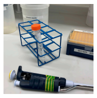
Protocol



NAME

96-well plate R3 desalt and clean up protocol for mass spec analysis

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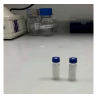
Protocol



NAME

Using the Thermo SPD1010 speedvac concentrator centrifuge for drying down peptides for LC-MS analysis

VERSION 1

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ronan o'cualain
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Protocol

NAME

Sample submission for LC-MS BioMS core facility at the University of Manchester

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