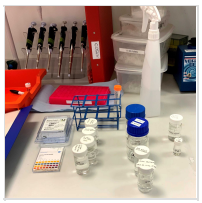

Aug 16, 2022

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

In 1 collection

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ABSTRACT

This protocol details the procedure of sample lysis and protein extraction using the Covaris LE220+ ultrasonicator.

ATTACHMENTS

iiaebptmp.docx

DOI

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PROTOCOL CITATION

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COLLECTIONS

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

KEYWORDS

Protein extraction, Covaris LE220+, Sample lysis, biological, clinical, sample preparation, LC-MS, ultrasonication, mass spectrometry, proteomics, microproteomics, tissue, cells, sonication

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PARENT PROTOCOLS

Part of collection

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

GUIDELINES

Initial assumptions and preparation:

- Allow approximately **00:30:00** for sample lysis and protein extraction.
- You may pre-book the use of the LE220+ machine beforehand on PPMS.
- If it is your first time using the LE220+, let us know at bioms@manchester.ac.uk, you will be provided with training, in which you will be provided with login rights to the machine and some basic processing methods to get you started.
- You have cells or tissue in Covaris tubes, **On ice** .

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MATERIALS TEXT

Locate the following buffers, consumables, and reagents:

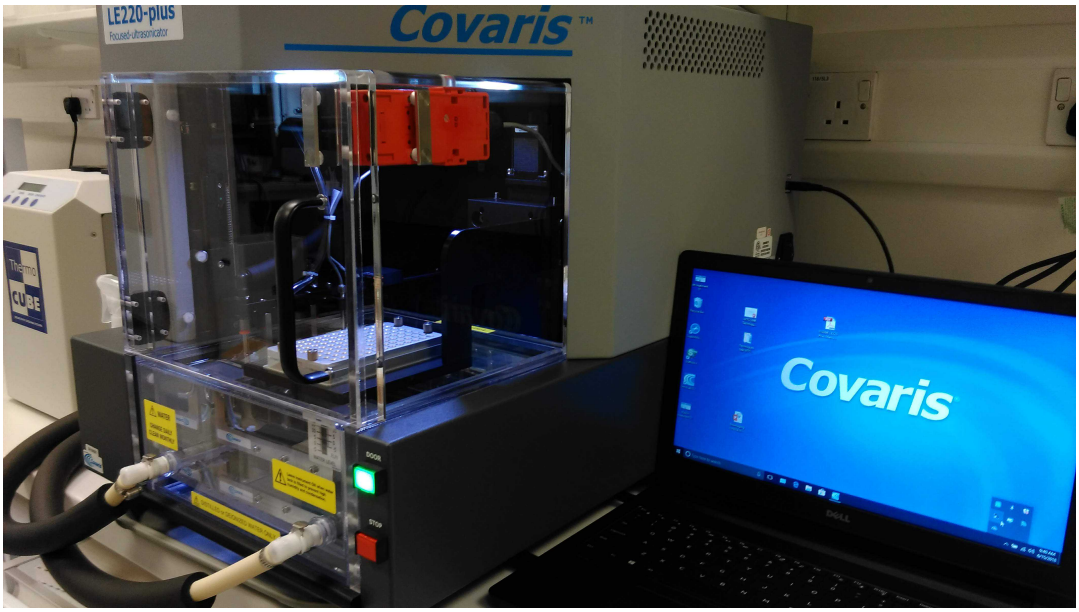
A	B
Location	Buffer/reagent
Fridge 02	N/A
Bench	2x stock of S-trap lysis buffer (10% SDS in 100 mM TEAB, pH 7.5) LC-MS grade water Eppendorf tubes
Freezer	N/A

SDS **Sigma Catalog #75746**

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

Identify the following equipment that you will use:

- p20 pipette, p200 pipette, p1000 pipette and pipette tips
- Stainless steel rack for LE220+ processing (500282 rack for 520045 tubes, 500452 rack for 520185 tubes) – Ask a member of BioMS staff for it.
- Spare Covaris tubes for making up the rows in the rack to either 8 per row for the 500282 rack, or 4 for the 520185 rack, check next to LE220+ machine.



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BEFORE STARTING

Initial preparation:

Before you begin:
Log onto the LE220+ and check that the “run” box in the top right hand corner is green. If not, it may be that the system is not at temperature. Click the “System status” box on the right hand side – if a red “X” is next to temperature, then you must wait until the system reaches the temperature that you have set the system to in your method before you begin. The default temperature for the system is **10 °C** .

Preparing samples and LE220+

1

You will need to dilute the S-Trap lysis buffer to a working stock concentration.

S-trap lysis buffer (10% SDS) is provided in the orange trays on bench.

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Using the 1mL pipette, add **500 µL** of 2x stock of S-Trap lysis buffer to **500 µL** of LCMS grade water.
This is the working stock concentration (5% SDS, **50 millimolar (mM)** TEAB pH **7.5**).

If you know that your samples are high in disulphide bonds, you may add **5 millimolar (mM)** of dithiothreitol (DTT) at this stage. Refer to the section on "reduction and alkylation" for instructions on how to prepare DTT.

2

Add S-Trap lysis buffer (5% SDS with **50 millimolar (mM)** TEAB pH **7.5**).

Do not freeze your samples once they contain buffer, it will cause the glass tube to crack.

2.1

If using the 520045 tubes, add approximately **50 µL** to **130 µL** of buffer, the final volume will depend on the amount of sample already present in the tube.

It is not recommended by Covaris to use **100 µL** in 520045 tubes, the headspace caused by the liquid to air ratio impacts on the performance of the instrument

2.2

If using the 520185 tubes, add up to **500 µL** of buffer, again this is dependent on the amount of sample that you wish to process.

3 Add the Covaris tubes to the respective stainless steel rack.

According to Covaris, all spaces in the row must be filled, there are spare tubes beside the machine for this purpose. Tubes are processed in rows on the width of the plate.

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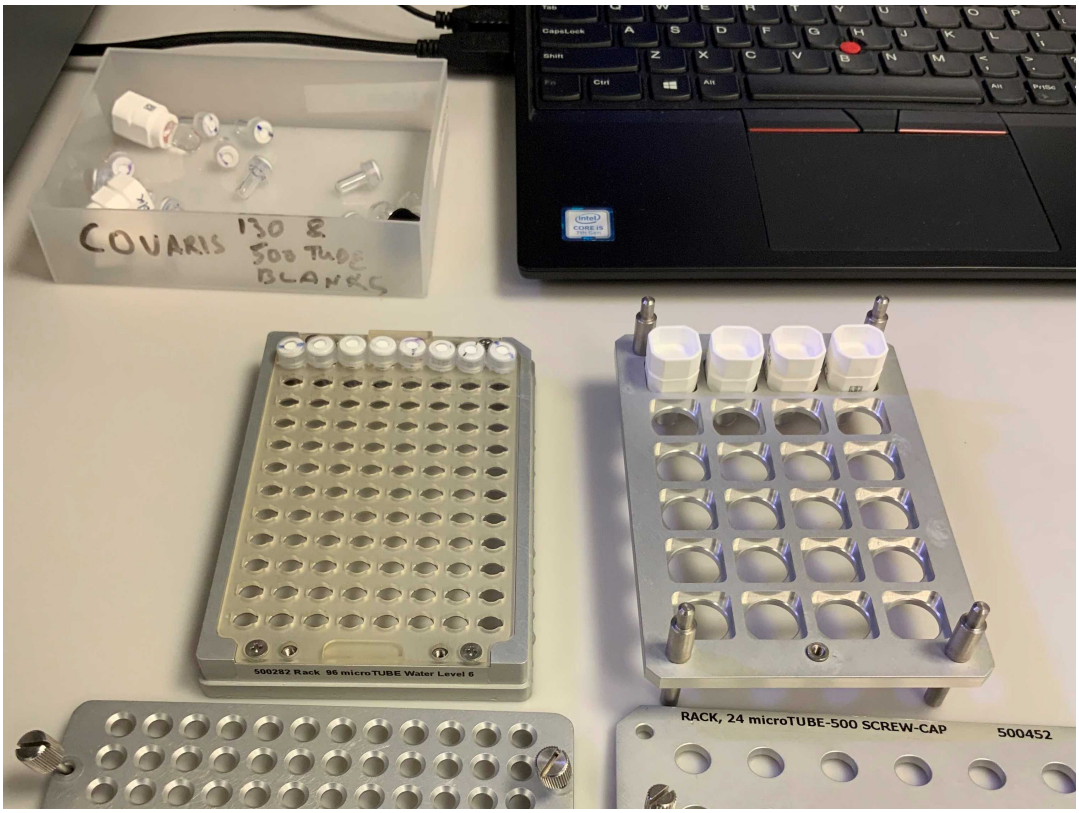

Covaris tubes in their racks - NB: sample tubes are processed in rows of either 4 or 8, with no gaps.

4 Clamp the lid on the rack finger-tight, and keep the samples in the rack **On ice** until you are ready for processing.

Sonolab 8.2 software

5 On initial training, we will provide you with a general method that should be sufficient to lyse your sample. At the end of the process, if you feel that your sample would benefit from more treatment, simply run the program again.

Log onto the LE220+ focussed ultrasonicator and check that the “run” box in the top right hand corner is green. Sonolab software controls the LE220+ system.

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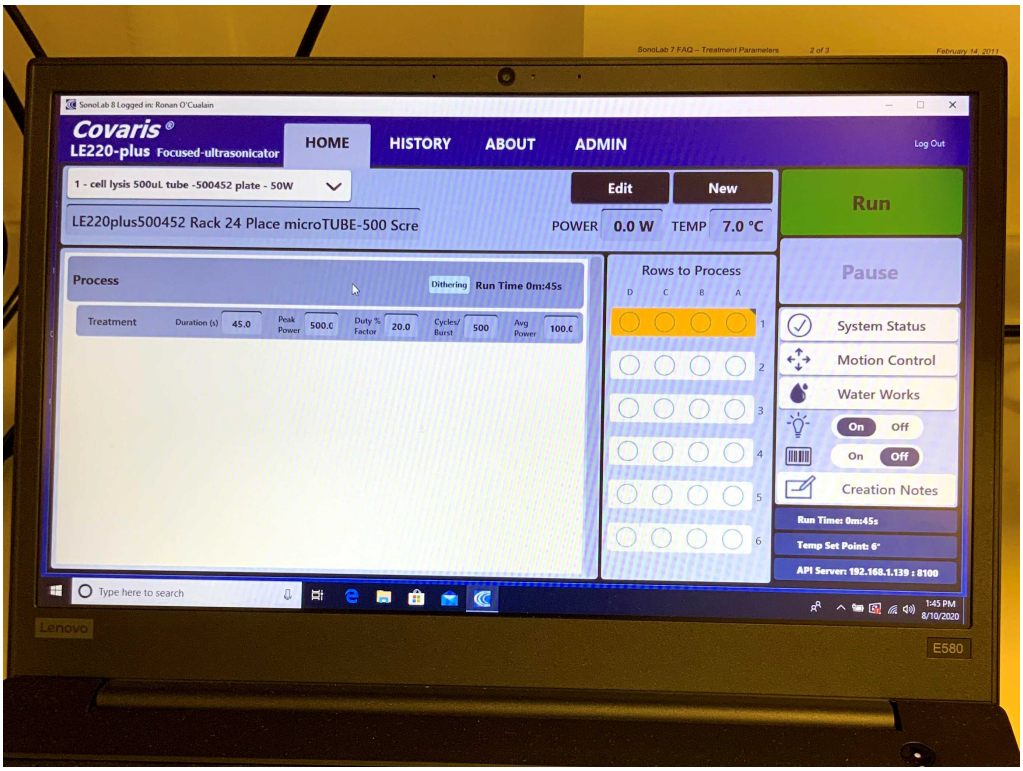
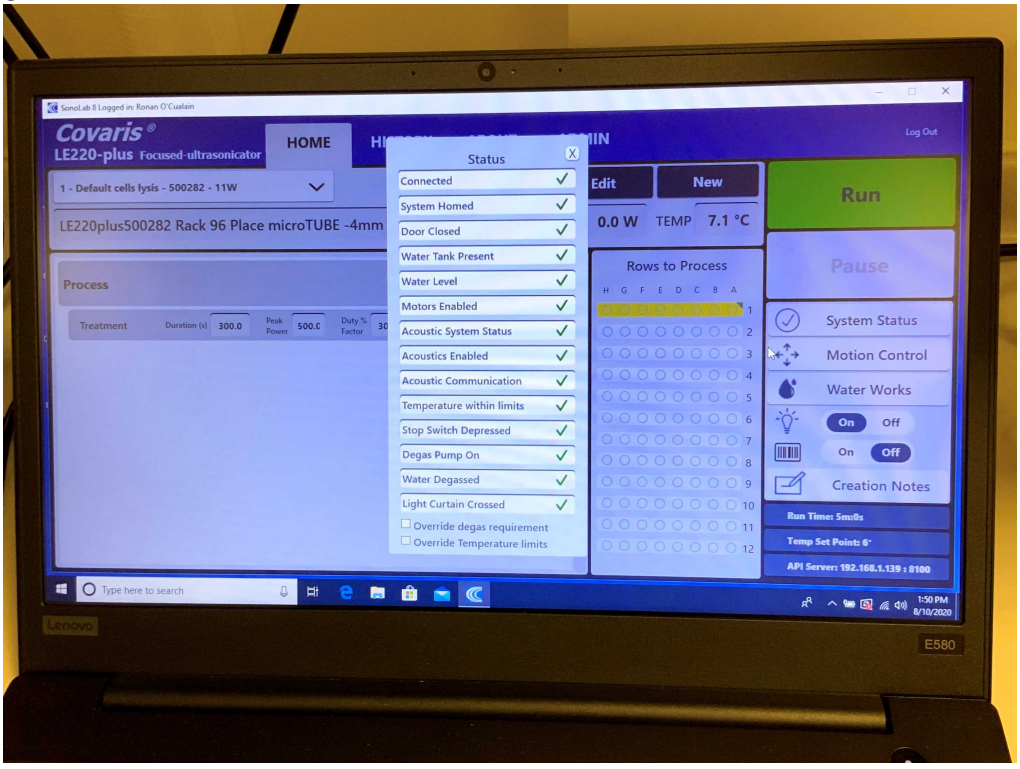


LE220+ focussed ultrasonicator Sonolab software.

If not, it may be that the system is not at temperature. Click the “System status” box on the right hand side.



Settings

– if a red “X” is next to temperature, then you must wait until the system reaches the

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temperature that you have set the system to in your method before you begin. The default temperature for the system is **10 °C** .

5.1 How will you know if your sample is ready?

With cell suspensions, the sample will turn from opaque to transparent, a sign that the cells have lysed.

5.2 

With tissue, after a quick centrifuge spin, you will see that your pellet is less than before.

6 Sonolab will automatically select the last method run by the user.

7 To select an alternative method, use the drop-down menu to load an alternative method, it is located on the HOME tab, in the top left corner.

8 To specify the rows of the plate that you wish to process, click “edit”.

9 This will open up the edit parameters page.

9.1 If you have two rows of tubes to process, click on the process in the left hand side.

This will allow the rows on the plate display on the right hand side of the page to be selected.

9.2 Click on the two rows that you wish to process.

When the LE220+ begins, it will process one row, then the next, in that order. The total processing time for what you specify in this page is displayed in the

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bottom right hand corner.

9.3 Click save and return to the main menu.

10 The Run method will remain grey and inactive until a method is selected and all necessary conditions are met.

10.1 On the right hand side, in the “Home” tab, take a look at the “System status” tab – if it is shaded red and contains a red X, then a starting parameter(s) has not been achieved – click on the button to see the issue.

Usually it is because the set temperature has not been reached. By default the LE220+ is set to **10 °C** .

10.2 If your method is set to **6 °C** , simply wait until the temperature has been reached.

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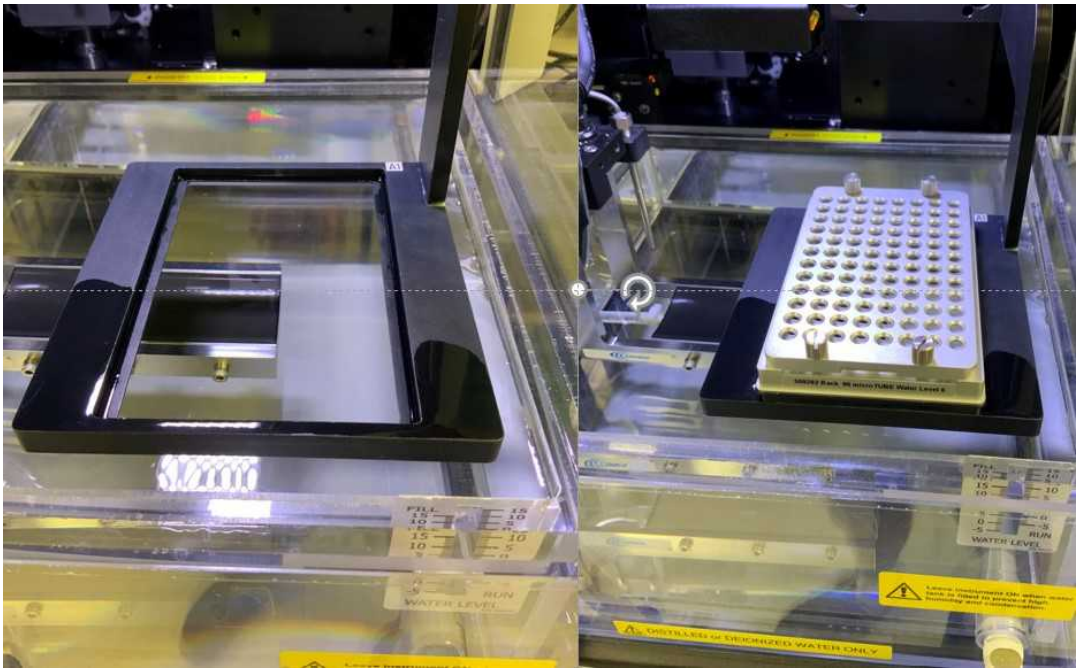
Running your method

11 Click on the “Motion Control” box, and click on “load”, the plate holder will move to the front of the LE220+ for plate loading.

12 Open the door of the LE220+ by simultaneously pressing the green door lock button while gently pulling the handle of the safety enclosure Perspex door.

13 Load the plate with the orientation that you specified in the method.



14 When the “Run” button is green, the selected method may be started. Click the “Run” button.

As the method runs, the graphic for the row under treatment flashes green, and the actual average incident power and temperature data are displayed.

When the method has finished, the message “Method Complete” is displayed. Click “OK”, then

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15 press the green door lock button and open the safety enclosure door. Remove the treated sample plate and place **On ice** .

16 Label **0.5 mL** or **1.5 mL** tubes for each 520045 or 520185 tube that you are processing, and transfer the lysed sample extract to the labelled tube.

You may now freeze your samples at this stage for storage, or proceed to reduction and alkylation.

17 If finished, **logout** of the Sonolab software and **return the rack** to the side of the LE220+ for the next user.

IMPORTANT: The Sonolab software should be always left ON, as by shutting it down, the default settings are triggered and the day to day maintenance settings are lost. If the software needs to be re-booted, contact a member of the facility.

Do not shut down the software!

Notes:

Treatments are defined by the following parameters: (For an in-depth explanation of these parameters, go to covaris.com)

- Treatment time.
- Cycles per Burst – the number of acoustic oscillations contained in each burst. The illustration below shows five cycles in each burst.
- Duty Factor – the percentage of active burst time in the acoustic treatment. The illustration shows a Duty Factor of 20%.
- Peak Incident Power – the power, in Watts, being emitted from the transducer during each burst.
- Average Incident Power - Peak Incident Power multiplied by the Duty Factor.

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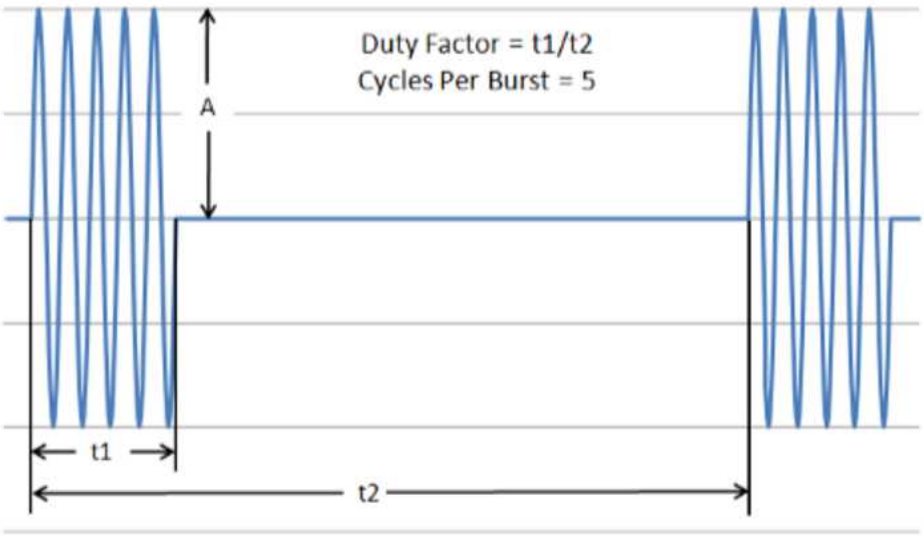

Figure 1 - Duty Factor and Cycles Per Burst

(For an in-depth explanation of these parameters, go to covaris.com)

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