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Cell lysis, detergent-free

Forked from Cell lysis, detergent-free

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1 Works for me dx.doi.org/10.17504/protocols.io.y4ffytn

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ABSTRACT

Detergents are generally not compatible with mass spectrometers, so this is a detergent-free method of cell lysis that is compatible with mass spectrometry. Since this protocol does not have a precipitation step, it saves time and minimizes sample loss as well.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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Growing Cells

1 Culture HeLa cells in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS, 20mM glutamine, and 1% PenStrep.

2 Original protocol uses $5x10^7$ cells per sample. Use $2x10^7$ cells. Use one 15cm Petri dish or 2-3 10 cm dishes. Each 10cm dish gives $8.8x10^6$ cells.

Preparing Solutions and Materials Needed

3 Ice Bucket

4 [M]2 Molarity (M) **tris at pH 8.5**

Add 1.211 g **tris-base** and 243 µl **concentrated HCl (37%)** up to a total of 5 ml **in Milli-Q water**

Calculations done with https://www.cytographica.com/lab/HHTris.html.

Tris-base is in NCE 438 chemical room on the top right doubles shelf

A stock conical tube of [M]2 Molarity (M) **tris** is stored on the bench.

5 [M]100 Milimolar (mM) **TCEP**

Add 250.187 mg **tris(2-carboethyl)phosphine** to 10 ml **Milli-Q water**

TCEP is located in NCE 436 -20C freezer **door** (currently the top shelf)

405 µl **aliquots** of [M]100 Milimolar (mM) **TCEP** have been made and are stored in Teesha's -20 °C **storage box**

6 [M]400 Molarity (M) **CAA**

Add 374.04 mg **2-chloroacetamide** to 10 ml **Milli-Q water**

CAA is located in NCE 438 chemical room on the "C" shelf

405 µl **aliquots** of [M]400 Milimolar (mM) **CAA** have been made and are stored in Teesha's -20 °C **storage box**

7 [M]50 Milimolar (mM) **NH4HCO3**

Add 197.4 mg **ammonium bicarbonate** to 50 ml **Milli-Q water**

Upscale or downscale volume needed. Up to 15 mL will be used for each replicate.

8 95 °C **heating block**

Pellet Cells

9 Using a 15 ml **conical tube** pellet cells for 00:05:00 **at 300 g**

Pellet in a tube a minimum size of 15 ml as this tube will be used through till the end of digestion.

10 Wash cells with 10 ml **cold PBS**

11 Pellet cells for 00:05:00 **at 300 g**

12 Carefully discard supernatant

13 Store cell pellet on ice

If not performing cell lysis immediately, the pellet can be stored at -80 °C **until further use.**

Cell Lysis

14 Resuspend the cell pellet(s) in 1.5 ml **ice cold Milli-Q water**

Perform the lysis and digestion in the 15 ml **conical tube** . The lysis volumes, sonification, and digestion require the larger tube volume.

15 Add **1.5 ml trifluoroethanol**

This step should be done in the fume hood.

1:1 water-TFE acts as a hypotonic aqueous buffer to lyse cells, eliminating the need for detergent. TFE helps protein solubility and denaturation; it readily evaporates, so removing it is easy.

TFE is located in the NCE 435 flammable cabinet.

TFE evaporates fast, so work quickly.

16 Cool for **00:10:00 on ice**

17 Mix the sample for **00:01:00 with a vortex**

18 Sonicate the sample for **00:02:00** with the Branson Digital Sonifier 250 at 30% amplitude in pulse mode (pulse ON for 0.2s and pulse OFF for 0.8s) with the tapered micro tip probe.

The Branson Sonifier is the cell distrupter of choice, however, the bench top water bath sonicator can be used for 10 minutes.

Reduction & Alkylation

19 Add **200 µl 2M tris** for a final concentration of **100 Milimolar (mM)**

20 Mix the sample for **00:00:05 with a vortex**

21 Add **400 µl 100 mM TCEP** for a final concentration of **10 Milimolar (mM)**

22 Mix the sample for **00:00:05 with a vortex**

23 Add **400 µl 400 mM CAA** for a final concentration of **40 Milimolar (mM)**

24 Mix the sample for **00:00:05 with a vortex**

25 Incubate in the heating block for **00:10:00** at **95 °C**

LysC Digestion

26 Dilute the sample to a total of **15 ml 50mM NaHCO3**

This is performed to dilute the TFE.

27 Measure the protein concentration with a NanoDrop (using SCOPES A205 Protein)

28 Calculate the total amount of protein that is desired to carry forward with the experiment. Keeping in mind that the final peptide concentration will be approximately 10-50% of the protein concentration at this step. Transfer this volume to a new tube. If small enough, transfer to a **2 ml lo-bind tube** .

29 Calculate how much LysC is needed for a **1 µg LysC** : **100 µg protein**

30 Add the calculated amount of LysC to the sample

LysC is in the **-80 °C freezer**

31 Incubate for **02:00:00** at **37 °C in the digestion incubator**

Trypsin Digestion

32 Calculate how much trypsin is needed for a **1 µg trypsin** : **100 µg protein**

33 Add the calculated amount of trypsin to the sample

Trypsin is in NCE 435 -20 freezer in the door, bottom shelf

34 Incubate for a minimum of **16:00:00** at **37 °C** **in the digestion incubator**

Second Trypsin Digestion

35 Calculate how much trypsin is needed for a **1 µg** **trypsin** : **100 µg** **protein**

A second trypsin digestion is optional. User digression is advised.

36 Add the trypsin to the sample

37 Incubate for **05:00:00** at **37 °C** **in the digestion incubator**

STAGE Tip

38 Proceed to the STAGE tip protocol