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Cell lysis, detergent-free

Forked from Cell lysis, detergent-free

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We use this protocol and it's working

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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.

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 5×10^7 cells per sample. Use 2×10^7 cells. Use one 15cm Petri dish or 2-3 10 cm dishes. Each 10cm dish gives 8.8×10^6 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

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

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

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

5 [M] 100 millimolar (mM) TCEP

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

Note

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

Note

405 µL aliquots of [M] 100 millimolar (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

Note

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

Note

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

7 [M] 50 millimolar (mM) NH4HCO3

Add 197.4 mg ammonium bicarbonate to 50 mL Milli-Q water

Note

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

> Note
>
> 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

> Note
>
> 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

> Note
>
> 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

Safety information

This step should be done in the fume hood.

Note

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.

Note

TFE is located in the NCE 435 flammable cabinet.

Note

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.

Note

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 [M] 100 millimolar (mM)

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

21 Add 400 µL 100 mM TCEP for a final concentration of [M] 10 millimolar (mM)

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

23 Add 400 µL 400 mM CAA for a final concentration of [M] 40 millimolar (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

> Note
>
> 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

Note

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

Note

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

Note

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