

Nov 01, 2019

Cell lysis, detergent-free



Forked from [Cell lysis, detergent-free](#)

DOI

dx.doi.org/10.17504/protocols.io.y4ffyt

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Protocol Citation: Teesha C Luehr 2019. Cell lysis, detergent-free. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.y4ffyt>

Manuscript citation:
<https://doi.org/10.1074/mcp.M114.047407>



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Protocol status: Working

We use this protocol and it's working

Created: March 12, 2019

Last Modified: November 01, 2019

Protocol Integer ID: 21351

Keywords: free method of cell lysi, free detergent, compatible with mass spectrometry, cell lysi, mass spectrometry, detergent, compatible with mass spectrometer, mass spectrometer, cell

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.

Troubleshooting



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 μ L 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 μ L 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 μ L 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) NH_4HCO_3

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 disrupter of choice, however, the bench top water bath sonicator can be used for 10 minutes.



Reduction & Alkylation

- 19 Add  200 μ L 2M tris for a final concentration of  100 millimolar (mM)
- 20 Mix the sample for  00:00:05 with a vortex
- 21 Add  400 μ L 100 mM TCEP for a final concentration of  10 millimolar (mM)
- 22 Mix the sample for  00:00:05 with a vortex
- 23 Add  400 μ L 400 mM CAA for a final concentration of  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