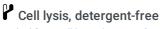


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Forked from Cell lysis, detergent-free

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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.
- 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

Molarity (M) tris at pH 8.5 □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.
DO Milimolar (mM) TCEP 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 δ -20 °C storage box
00 Molarity (M) CAA □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 8 -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	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 8-80 °C until further use.			
Cell L	ysis			
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.
	ction & Alkylation
19	Add 200 μl 2M tris for a final concentration of [M]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 [M]10 Milimolar (mM)

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

23	Add $=400~\mu l$ 400 mM CAA for a final concentration of [M]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 8 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 8-80 °C freezer
31	Incubate for $& 02:00:00$ at $& 37$ °C in the digestion incubator
Tryps	in Digestion
32	Calculate how much trypsin is needed for a $\ \ \ \ \ \ \ \ \ \ \ \ \ $
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 \odot 16:00:00 at $~8$ 37 °C in the digestion incubator			
Seco	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			

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