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| STANDARD OPERATING PROCEDURE |
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| **Title: Trypsin digestion of Cell Lysate** |
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# Purpose

The purpose of this document is to describe the enzymatic digestion and subsequent desalting of a cell lysate to prepare proteomic samples that are compatible with mass spectrometry analysis.

# Scope

This procedure may be used to reduce, alkylate, proteolyze, and desalt cell lysate samples.

# Responsibilities

It is the responsibility of person(s) performing this procedure to be familiar with laboratory safety procedures. The interpretation of results must be done by a person trained in the procedure and familiar with such interpretation.

# Equipment

* 37**°**C heat block

# Materials

* Sep-Pak C18 plus light cartridge, 130 mg Sorbent per cartridge, 55-105 µM particle size (Waters; cat. # WAT023501)

# Reagents

* SigmaUltra Iodoacetamide (Sigma-Aldrich, I1149-5G)
* DL-Dithiothreitol (Sigma-Aldrich; cat. # D9779-10G)
* Trypsin Gold (Promega, V5280)
* Tetraethylammonium bicarbonate (CH3CH2)4N(HCO3) (Sigma-Aldrich; 1268-25G-F)
* Trifluoracetic Acid (Sigma-Aldrich; cat. # T6508-500ML)
* Water: Optima LC/MS-grade (Fisher Scientific; cat. # W6-4)
* Acetonitrile: Optima LC/MS-grade (Fisher Scientific; cat. # A955-4)
* Formic Acid: Pierce Formic Acid, LC-MS grade (Pierce; cat. # 28905)

# Solutions

* 200 mM Iodacetamide (IAM) Stock Solution
	+ Suspend 36.8 mg of Iodoacetamide into 1 mL of HPLC water. Mix until fully dissolved.
	+ Prepare immediately prior to use. Keep out of light.
* 100 mM Dithiothreitol (DTT) Stock Solution
	+ Resuspend 18.4 mg in 1 mL HPLC water. Mix until fully dissolved.
	+ Prepare immediately prior to use.
* Trypsin Stock Solution
	+ Resuspend 20 µg lyophilized trypsin in 0.2 mL HPLC water (final concentration of enzyme after suspension of 0.1 µg/µL)
* 50 mM Tetraethylammonium bicarbonate (TEABC) (CH3CH2)4N(HCO3)
	+ Dilute 1M TEABC stock solution 1:20 (vol/vol) with HPLC water
* 20% Trifluroacetic Acid (TFA)
	+ Dilute TFA stock solution 1:5 (vol/vol) with HPLC water

# Procedure

1. Thaw cell lysate in 8M Guanidine HCL on ice for processing. Once thawed completely, vortex to mix thoroughly.
2. Protein Reduction and Alkylation
	1. Add 100 mM DTT stock solution to an aliquot of thawed cell lysate in sufficient volume as to yield to a final concentration of 10mM DTT.
	2. Incubate cell lysate and DTT mixture at 60**°**C for 30 min to reduce protein disulfide bonds. Allow mixture to equilibrate to room temperature.
	3. Add 200mM IAM stock solution to lysate mixture in sufficient volume as to yield a final concentration of 20 mM IAM.
	4. Incubate mixture at room temperature for 20 min in the dark to alkylate thiols of protein cysteines.
3. Protein Trypsinization
	1. Add 50 mM TEABC to protein mixture in sufficient volume so as to dilute the initial 8M Guanidine HCL storage buffer concentration (see “SOP: Cell lysate preparation” for solution recipe) to a concentration below 2M. Add additional TEABC as required until the pH of the diluted solution is between 7.0 and 8.0.
	2. Add sufficient volume of the trypsin solution to each digest to achieve a 1:20 enzyme:protein substrate ratio.
	3. Incubate protein solution at 37**°**C with mixing for 16 hours to facilitate proteolysis.
4. Acidification of Digested Cell Lysate
	1. To quench trypsin reaction, dilute peptide digest in 20% TFA so that the pH of the final solution is under 3 and the final concentration of TFA is 1.0%.
	2. After acidification, allow precipitate to form by incubating digest on ice for 15 min.
	3. Centrifuge acidified peptide solution at 13,200xg (or max speed) for 10 min at 4**°**C to remove precipitate. Transfer peptide-containing supernatant into new tube.
5. Prime Sep-Pak Cartridge
	1. Connect a 10 cc reservoir to the short end of the Sep-Pak cartridge.
	2. Condition the column with 5.0 mL 100% ACN.
	3. Wash column with additional 5.0 mL ACN, 0.1% TFA.
	4. To equilibrate column, wash column sequentially with 1 ml, 3 mL, 5 mL, and 6 mL of 0.1% TFA.
6. Peptide Purification
	1. Load acidified digest prepared in Step 4c onto equilibrated Sep-Pak cartridge.
	2. Wash column sequentially with 1ml, 3mL, 5 mL, and 6 mL of 0.1% TFA.
	3. Place Sep-Pak column above new 15 mL falcon tube to collect eluent.
	4. Add 3.0 mL of 0.1% TFA, 40% ACN to Sep-Pak column to dilute desalted peptides off Sep-Pak column, collecting eluent. Repeat, collecting both eluents in a single tube.
	5. Lyopholize peptide solution to dryness to ensure TFA has been removed from the peptide sample.
	6. Samples can be stored lyophilized at -80**°**C until ready for PRM analysis.
7. Reconstitute samples prior to LC-PRM
	1. Re-constitute dried and desalted peptide digests with 0.1% formic acid to achieve a concentration of 2mg/mL of peptide.
	2. Vortex sample to resuspend thoroughly and transfer desired peptide amount to microcentrifuge tube or 96-well plate.

# Referenced Documents

* SOP: Cell lysate preparation