HeLa quality control sample preparation for MS-based proteomics

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Abstract

Cell lysis and digestion protocol of HeLa cell line for quality control measurements of mass spectrometry-based proteomics measurements.

Introduction

In solution digest of human proteome sample for HeLa quality control runs of mass-spectrometers used at Novo Nordisk Foundation Center for Protein Research. The protocol has no specific author and is adapted by individual researchers at the institute.

Reagents

6M Guanidine Hydrochloride (MW: 95.53g/mol) – 5.73g
5mM TCEP (stock: 0.5M) – 100x – 100ul
10mM CAA (stock: 550mM) – 55x – 182ul
100mM Tris pH 8.0 (stock 1M) – 10x – 1ml
2ml of Bradford

Equipment

Procedure

Cell lysis

1. Prepare 10ml Lysis buffer fresh

6M Guanidine Hydrochloride (MW: 95.53g/mol) – 5.73g
5mM TCEP (stock: 0.5M) – 100x – 100ul
10mM CAA (stock: 550mM) – 55x – 182ul
100mM Tris pH 8.0 (stock 1M) – 10x – 1ml
Water ~ 5ml

1. Heat lysis buffer to 99°C
2. IF STARTING FROM PELLET – SKIP STEP 4 and 5.
3. Aspirate media completely from the plate
4. Wash with 10 ml of PBS twice
5. Add 1000ul lysis buffer (and scrape cells off the plate) and collect in a 15 ml falcon tube
6. Heat lysate at 99°C for 10min in the heating block while shaking
7. Sonicate with microtip probe for 2min with pulses of 1sec ON, 1sec OFF at an amplitude of 50%.

**Measure protein concentration using Bradford Dye Reagent (in common fridge)**

1. Prepare protein standards in the range 0, 1, 2, 4 and 8ug/ml using BSA
2. Mix 1ml of Bradford and the appropriate volume of BSA (stock: 1mg/ml)
3. Mix 1ml of Bradford and 1ul of your protein sample
4. Prepare a blank sample with 1ul lysis buffer
5. Vortex the samples gently and incubate for 5min
6. Measure the absorbance at 595nm for the standards and the sample
7. Calculate the protein concentration by plotting a standard curve of the absorbance vs. protein sample concentration

**Digestion**

1. Digest lysate with LysC: protein ratio 1:100 (w/w) (stock: 0.5ug/ul) and incubate shaking at 25°C for 60min (O/N) (5000ug = 100ul)
2. Dilute to minimum 2M Guanidine hydrochloride with 25mM Tris pH 8.5 (40x from stock)
3. Digest with trypsin: protein ratio 1:100 (w/w) (stock: 0.5ug/ul) and incubate at 37°C shaking O/N at 300rpm (5000ug = 100ul)
4. Stop the digest by quenching with TFA (pH should be reduced to ~2 by adding 1/10 volume of 10% TFA) Check with pH strip (NB: chemical hood!!)
5. Upconcentrate on Sep-Pak

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