Imperial College London

MULTI-'OMICS ATLAS PROJECT

March 2023

Protein extraction from fresh frozen human tissue

(https://www.nature.com/articles/s41598-021-87003-6#Sec11)

PROTOCOL

Protein Extraction from fresh frozen human brain tissue for LC-MS.

Before starting:

1) Take previously prepared samples from -80 and keep on dry ice until ready to begin
2) Cool the microcentrifuge to 4 degrees.
3) Take ethanol from -20 and keep on ice
4) Take ammonium bicarbonate from 4 degrees (cold room) and keep on ice.
5) Take milliQ water from 4 degrees (cold room) and keep on ice.
    a. Add 1mL ice-cold 70% ethanol (v/v) (keep on ice), with end-over-end rotation at 4°C for 2 minutes.
    b. Centrifuge at 10,000xg for 2 min at 4°C and remove the ethanol.
    c. Repeat b-c for a total of 3 washes.
    d. Add 1mL ice-cold water, with end-over-end rotation at 4°C for 2 minutes.
    e. Centrifuge at 12,000xg for 2 min at 4°C and remove the water.
    f. Repeat e-f for a total of 3 washes.
    g. Add 1mL ice-cold 50mM ammonium bicarbonate, with end-over-end rotation at 4°C for 2 minutes.
    h. Centrifuge at 12,000xg for 2 min at 4°C and remove the AmBic,
    i. Repeat g-h for a total of 3 washes.
    j. Allow the samples to air dry on ice for 5 minutes (keep the lid of icebox closed).

6) Protein extraction:
    a. Take PMSF out of -20 and add 0.1mM PMSF to the lysis buffer.
    b. Return 200mM PMSF to -20C immediately.
    c. Add 150ul lysis buffer to each sample.
    d. Sonicate with a homogeniser with an up and down motion for 30sec on ice (see bubbles forming).
    e. Centrifuge for 15 minutes at 12,000xg and retain supernatant
    f. Transfer supernatant (avoid the whiteish debris and pellet) to new Eppendorf and store at -80C.
    g.
7) Freeze and then send to facility to (briefly):
    a. Perform protein assay
    b. With 10-20ug of protein:
    c. Dilute to 1M urea
    d. Add 10mM TCEP, 40mM Chloroacetamide, trypsin at 1:50 (digest the protein at specific site, trypsin has the largest database)

e. Incubate at 37°C for 16 hours (o/n).
f. Acidify to 0.5% TFA (v/v) and desalt.

8) MS = nanoLC-HDMS^e with 90-minute gradient. Data analysis in Progenesis QI for Proteomics.

SOLUTIONS

Ammonium Bicarbonate Solution	**1 SAMPLE**	**6 SAMPLES**	**Final Concentration**
Ammonium Bicarbonate powder	19.8mg	118.59mg	50mM
Water	4mL initially add the rest after solid is dissolved	20mL initially add the rest after solid is dissolved	-
Total Volume	5mL	30mL	-

Lysis Buffer	**1 SAMPLE**	**6 SAMPLES**	**Final Concentration**
Ammonium Bicarbonate powder	26.35mg	158.12mg	1M
Urea powder	160.16	960.96mg	8M
Water	200µL initially and add the rest after solid is dissolved	1.5mL initially and add the rest after solid is dissolved	-
Total Volume	330µL	2mL	-

70% Ethanol	**1 SAMPLE**	**6 SAMPLES**	**Final Concentration**
Molecular grade ethanol	3.5mL	21mL	70%
Water	1.5mL	9mL	-
Total Volume	5mL	30mL	-

For PMSF (200mM aliquots at -20), add 1ul to 2mL lysis buffer (0.1mM)