Dimethyl Labeling for Global Proteome Comparison

Materials:

Lysis buffer with protease inhibitor cocktail

Acetone -20°C

8M urea in 50mM TEAB (pH 8.0, made fresh, never heated)

TCEP or DTT

Iodoacetamide

Trypsin

Pure Formic Acid

80% acetonitrile (CAN) containing 0.1%TFA

0.1% TFA

80% ACN containing 0.1%TFA

100 mM TEAB

4% (vol/vol) formaldehyde - CH_2O (light label), CD_2O (intermediate label)

0.6 M $NaBH_3CN$

1% (vol/vol) Ammonia solution

Day 1:

1) Lyse cells with lysis buffer (Make sure includes protease inhibitor cocktail).
2) Centrifuge at 4˚C 13,000 rpm for 20 minutes to remove cell pellet (unsolubilized protein)
3) Transfer supernatant to new Eppendorf tube for acetone precipitation.
   Target to precipitate ~100µg of protein.
4) Perform acetone precipitation with 5x volume of ice-cold acetone (kept at -20 °C)
5) Precipitate at -20 °C overnight

Day 2:

1) Spin @13,000rpm, 4 °C, 10 min, remove the supernatant, and slowly wash the pellet once with ice cold acetone (spin again). Remove the supernatant and keep the pellet.
2) Note: remainder of protocol assumes protein concentration is 100 µg (If pellet too large, i.e. too much protein, you will have problems resolubilizing it. It is VERY important the entire pellet is solubilized.)
3) Re-suspend the pellet in 100uL of 8M urea in 50mM TEAB (pH 8.0, must make fresh and never heat!) and vortex or sonicate to solubilize the pellet, incubate with shaking at 37 °C for 30 minutes;
4) Run protein assay (BCA assay) to quantify protein amount for each sample.
5) Add TCEP or DTT to 10 mM and shake for 45 min at 37 °C. You can add TCEP/DTT to the solubilization buffer if you are using a reducing agent compatible BCA assay.
6) Add iodoacetamide to 55 mM, shake for 45 min at 37 °C;
7) Dilute to final 1M urea (dilute with 25mM TEAB) and digest with trypsin (1:40) shaking overnight at 37 degree.

Day3.

1) SPE clean-up
2) Acidify solution to 1% (v/v) with pure formic acid;
3) SPE clean-up with SPE cartridge (use the size for 200-500 µg protein):
    a. Wash the cartridge with 1ml of 80% acetonitrile (CAN) containing 0.1%TFA
    b. Wash with 1.2ml of 0.1% TFA X 2 times
    c. Load sample
    d. Wash sample with 1.5ml of 0.1% TFA
    e. Elute with 800 µL of 80% ACN containing 0.1%TFA and collect in Eppendorf tubes. (protein samples can be aliquot to several tubes with equal amount of solvent).
    f. Speedy-vac dry for 20 min to remove partial ACN and then freeze dry samples. Samples can be stored at this stage.

Day 4:

Labelling process

1) Samples have to be free of primary amines (SPE prior or acetone precipitation for protein)
2) This is assuming you have 100 µg of protein.
3) Reconstitute samples in 400 µl of 100 mM TEAB (<25ug protein)
4) Check pH is between 5-8.5
5) Aliquot 100 µl of sample to new Eppendorf tube.
6) Add 4 µl of 4% (vol/vol) formaldehyde - CH_2O (light label), CD_2O (intermediate label.)
    a. Norm formaldehyde = 2.16 µL + 17.84 µL H_2O
    b. Heavy formaldehyde = 4 µL + 16 µL H_2O
    c. Mix and spin briefly
7) Add 4 µl of 0.6 M $NaBH_3CN$ to samples to be light and intermediate labeled.
    a. 3.76 mg in 100 µL (need to make only 100 µL or less)
8) Incubate in a fume hood for 1 hour at RT (15-22 °C) while mixing using a bench top TT mixer.
9) Add 16 µl of 1% (vol/vol) Ammonia solution (Fume hood.)
    a. Ammonia solution stock = 25%
    b. 40 µL stock + 960 µL H_2O
10) Mix briefly and spin solution down (fume hood.)
11) Add 8 µL of formic acid (neat) to further quench reaction (fume) – do this on ice.
12) Mix differentially labelled samples.
13) Analyse differentially labelled samples using MS.

Adapted from Boersema, *et al*. *Nature Procotol*. 2009.