HepG2_FN3K Sample Preparation Protocol

Description

Protocol adapted from SECIM SOP: NMR sample prep of cells, effective 11/24/2014

Original protocol cited for approx 6 million cells.

Materials and Reagents

PBS buffer (15 mL x # of plates)

Aspirator

Dry ice

Liquid nitrogen

Cell scraper (one per plate)

Refrigerated centrifuge

Extraction solvent (Methanol:Water, 80:20 (v/v) UPLC or LC-MS grade)

Speed-vac concentrator

3mm NMR tubes

100 mM sodium phosphate buffer in D_2O, pH 7.4

Includes: 1/3 mM Sodium trimethylsilylpropanesulfonate (DSS-D6)

3 mM sodium azide

Sample Collection and Extraction Procedure

Rapid quenching of metabolic reactions is required to minimize the occurrence of unwanted metabolic stress and ‘run-on’ reactions.

Steps

1	Wash the monolayer three times with 5 ml cold PBS.
2	Add ice cold extraction solvent to plate, scrape cells, and transfer to a tube. (10 cm plate: 1.0 ml extraction solvent)
3	Freeze tubes immediately in liquid nitrogen and store at -80°C until extraction.
4	Thaw tubes on ice. (Approx 1 hr)
5	Vortex tubes well twice for one minute intervals.
6	Pellet the debris by centrifugation on highest speed for 15 min at 4°C.
7	Transfer the supernatant to a clean tube and label.
8	Keep debris tube as well for protein concentration determination/sequential extraction.
9	Evaporate the solvent from extracts using speed-vac instrument until dry, approx. 4-10 hrs (Labconco).
10	Store dried samples at -80°C until NMR/mass spec analysis.

Steps 1-3 Performed by Sami and Kannan Lab.

Harvested cells transfered on dry ice to Edison Lab 2/8/18.

Rest of extraction procedure performed by MBC in Edison Lab.

NMR Preparation

Dried extracts resuspended in 200 uL sodium phosphate NMR buffer in randomized run order. Buffer blanks loaded directly into tubes before first sample and after last sample resuspended. All steps performed on ice/4°C.

1. After addition of buffer, samples vortexed for 1 min
2. Spun down in microcentrifuge for 15 sec.
3. 180 uL transferred into 3mm NMR tubes.
4. 15 uL of remaining sample combined to form internal pooled samples, loaded last.
5. NMR tubes kept on ice/4°C until transfer to SampleJet for analysis