20170322 2HG extraction procedures for cell matrix

Samples

Castro-Lowenstein, EX00705, N=24 cells, LOC 0000
, EX00 , N= cells, LOC0000

Extraction Solvent

_____ mL of 7:2:1 (Methanol: H_2O: Chloroform) +_____ µL of 100µM ^{13}C Hydroxyglutaric acid [Final 2µM]

DATAN: diacetyl-l-tartaric anhydride- 50mg/mL in Dichloromethane-**acetic acid** (4:1- 5 parts)

Standard Mix stock (STD) and Internal Standard stock (IS)

Hydroxyglutaric acid ^{13}C 100µM

Standard Preparation (STD Mix)--LCMS

STD [Final-µM]	MeOH (µL)	$ChCl_3$ (µL)	H_2O (µL)	2HG STD mix (µL) of 25µM D + L	Hydroxyglutaric Acid 100µM
STD 0 [0 µM]	140	20	40	0	4
STD 1 [0.06 µM]	140	20	39.5	.5	4
STD 2 [0.125 µM]	140	20	39	1	4
STD 3 [0.625 µM]	140	20	35	5	4
STD 4 [1.25 µM]	140	20	30	10	4
STD 5 [2.5 µM]	140	20	20	20	4
STD 6 [5 µM]	140	20	0	40	4
STD A	100	20	0	80 D-2HG	4
STD B	100	20	0	80 L-2HG	4

Sample Preparation

1) Place all samples on wet ice until extraction procedure has been completed.
2) While over wet ice **add 0.6ml Extraction solvent** containing Internal Standards (ISs) samples.
3) Sonicate with probe for 5-10secs set to 20% Duty cycle and 2 output, repeat vortex.
4) Keep eppendorf tubes at **4°C for 10min** to allow complete extraction, remove from 4°C repeat vortex.
5) **Centrifuge** all tubes at 14,000RPM for 10min in 4°C.
6) **Create a pooled** sample by transferring equal volumes from each sample to an autosampler vial.
7) **Transfer 200µL** of supernatant to an autosampler vial take to dryness using speed vac at 55° Aqueous for ~1 hr.
8) **Add 50µL** of 50mg/mL DATAN, cap and **incubate** at **75°C** for **30 min.**
9) **Cool vials and dry** by continuous N2 flow at RT°C for ~ 1 hr.
10) **Reconstitute** samples in 100µL of LC grade H2O, vortex, and transfer to insert.

LC–MS Analysis

QQQ- method: 2HG 6490 in CR methods folder
Column: Waters HSS T3 C18 50mm column.
MP A: 2mM Ammonium Formate in H_2O, pH ~3.3-4.01, adjust with LC-MS grade Formic Acid
MP B: 100% ACN with 0.1% Formic Acid

***Observe “L” and “D” peak in standards before deciding pH and Ammonium formate concentration of MP.

D = 363.2 → 147.2

L = 368.2 → 151.2

Notes/Observations