**15DEC2015: 2HG extraction procedure for cell matrix**

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| **Samples** |

Castro, EX00515, N=16 cell (plates/ pellets), LOC 0000 and Stegh, EX00518, N=70 cell(plates/ pellets), LOC0000

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| **Extraction Solvent** |

\_\_\_\_\_ mL of 7:2:1 (Methanol: H2O: Chloroform) +\_\_\_\_\_ µL of 200µM 13C Gly/TCA IS ([final] 2µM), \_\_\_\_\_µL of 100µM 13C α-KG([final] 2µM)

**(0.5ml for 6cm plate, 1.5ml for 10cm plate/ .5mL to pellet/ .5mL to filter paper)?????**

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

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

**GLY/TCA IS**: 13C 100µM- need to make more

**13C α-KG-** need to make more?

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| **Standard Preparation (STD Mix)--LCMS** |

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| STD [Final-µM] | MeOH (µL) | ChCl3 (µL) | H2O (µL) | 2HG STD mix (µL) | 13C4 α-KG mix (µL) | GlyTca 13C IS mix (µL) |
| STD 0 [0 µM] | 132 | 20 | 40 | 0 | 4 | 4 |
| STD 1 [0.06 µM] | 132 | 20 | 39.5 | .5 | 4 | 4 |
| STD 2 [0.125 µM] | 132 | 20 | 39 | 1 | 4 | 4 |
| STD 3 [0.625 µM] | 132 | 20 | 35 | 5 | 4 | 4 |
| STD 4 [1.25 µM] | 132 | 20 | 30 | 10 | 4 | 4 |
| STD 5 [2.5 µM] | 132 | 20 | 20 | 20 | 4 | 4 |
| STD 6 [5 µM] | 132 | 20 | 0 | 40 | 4 | 4 |
| STD A | 132 | 20 | 0 | 40 D-2HG | 4 | 4 |
| STD B | 132 | 20 | 0 | 40 L-2HG | 4 | 4 |

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| **Sample Preparation** |

1. Place all samples on wet ice until extraction procedure has been completed.
2. While over wet ice **add 0.5ml Extraction solvent** containing Internal Standards (ISs) samples.
3. Sonicate with probe for 5-10secs set to 4 Duty cycle and 4 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 45° 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.

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| **LC–MS Analysis** |

QQQ- method:

Column: Waters HSS T3 C18 50mm column.

MP A: 2mM Ammonium Formate in H2O, pH ~3.3, adjust with LC-MS grade Formic Acid

MP B: 100% ?

GCMS: DB5MS 30x0.25x0.25 column

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| **Notes/Observations** |