LOCASALE LAB PROTOCOLS

Suspension Cell Sample Preparation

1. In the end of treatment, collect 1-4E6 cells into Eppendorf tube. If cell sorting is required, collect cells into FACS sorting buffer (FBS + PBS). *Note: Cell number can be reduced to E5 or even high E4, for high abundant metabolites, such as intermediates in glycolysis, TCA or amino acids, but for SAM or SAH, E5 is required. For cell culture samples, prepare 3 replicates for each condition.*

2. Spin down at 300 rcf for 5 min at 4 ^{o}C.

3. Immediately aspirate media or buffer. *Note: Aspirate residue media or buffer AS MUCH AS POSSIBLE SINCE THE CONSISTENCY OF THIS STEP WILL AFFECT SENSITIVITY AND REPRODUCIBILITY. 10-20ul leftover or so is fine, but the less the better quality of the data.*

4. Cell wash (Optional step): add 1ml ice cold fresh medium or 0.9 % NaCl (900mg/100ml LC grade water), pipette up and down, and repeat step 2 and 3. *Note: Wash step is required if your medium contains high contents of salts (especially when you use FACS sorting buffer), or* i*f you want to only measure intracellular metabolites.*

5. Place samples on dry ice and immediately add 1ml 80% MeOH/H_2O (both HPLC grade, precooled in -80^{o}C freezer for at least 1hr) to cell pellet. Leave samples in -80 °C for 15min to further inactivate enzymatic activities.

6. Remove samples from -80 ^{o}C freezer, place on regular ice and vortex couple of times (30s each time) to achieve a good extraction.

7. Centrifuge at 20 000 rcf for 10 min at 4 ^{o}C (to precipitate proteins, oligonucleotides, etc).

8. Split supernatant into two tubes. *Note: we will only analyze one tube in LC-MS. The other tube is used as a backup and shipped to us for future analysis or in case anything goes wrong with the analysis on our end (e.g. instrument glitch during the run, etc).*

9. Dry all samples using speed vac and store at -80^{o}C freezer.

10. Keep samples in sample box and ship to us on dry ice.

Prepared by Locasale Research Group (April 2017)