**LOCASALE LAB PROTOCOLS**

## Cell Culture Sample Preparation

1. Grow cells in 6-well plate (bigger plate or dish is not acceptable) to 50% - 80% confluence. NOTE: try your best to extract the same number of cells when comparing across a set of conditions (e.g. WT vs. KO). Let us know if this is not possible and we can think of alternatives.
2. Tilt the plate, and immediately aspirate medium at room temperature (about few seconds per well). TRY TO REMOVE THE MEDIUM AS MUCH AS POSSIBLE SINCE THIS WILL AFFECT SENSITIVITY AND REPRODUCIBILITY.
3. Immediately place the plate on dry ice, and add 1 mL 80% methanol/water (both HPLC grade) (pre-cooled in -80$^{o}$C for at least 1hr).
4. Transfer the plate to a -80$^{o}$C freezer and leave it there for 15min to further inactivate enzyme activities.
5. Remove the plate from -80$^{o}$C freezer and put it on dry ice, scrape cells into extraction solvent.
6. Transfer the whole cell extract to a new Eppendorf tube placed on ice.
7. Centrifuge at 20 000 rcf for 10 min, 4$^{o}$C.
8. Transfer the supernatant into two tubes, save one tube as backup (roughly 450 µL per tube). Discard the old tube containing the pellet of debris.
9. Dry with a speed vacuum at room temperature, which will take about 1.5 to 2 hrs for 450 µL solvent. Note: The dry pellet should be small (smaller than the volume of 5 µL liquid) and pink color due to the residual medium. Vacuum times vary depending on the version you're using.
10. Store dry pellet in -80 $^{o}$C freezer for further LC-MS analysis.

**NOTE: The Stable Isotope Tracing Assay should be conducted on a case-by-case basis.**

Prepared by Locasale Research Group (September 2016)