LOCASALE LAB PROTOCOLS

Additional Notes on Sample Preparation

1. PBS, EDTA, Tris, OCT, detergents, HEPES, nonvolatile acids/bases (i.e. Anything that forms ions and will get injected into the mass spec) are incompatible with our mass spec. e.g. Washing cells with PBS or retaining any residual media in a cell culture extract will dramatically lower sensitivity, decrease reproducibility, and in extreme cases potentially damage our instrument.

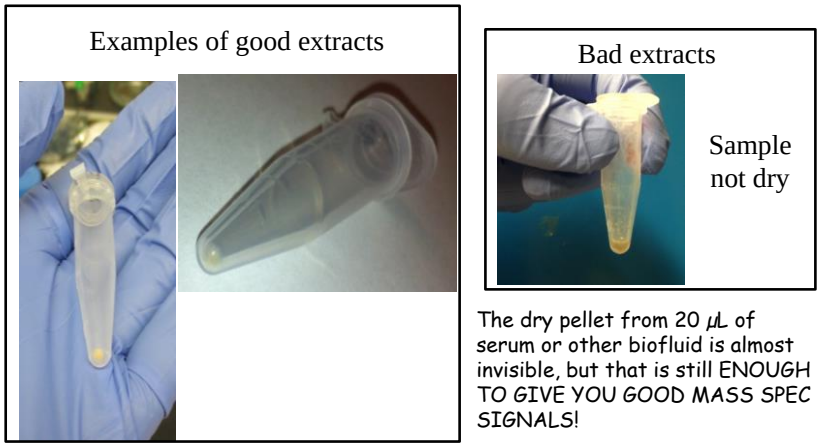
   Particularly, our ion trap based mass spectrometer is different from other types of mass spec. In general, salts are never good for mass spec analysis but our instrument is more sensitive to salt content even more so than other mass spectrometers, for several reasons including:

   a). The enhanced ion focusing system has higher sensitivity through improved efficiency in ion transmission, and meanwhile allows more salts going into the system too.

   b). The Ion trap can only hold a fixed amount of ions, so if there are lots of salts, they will occupy the trap and kick out the molecular ions of interest of relevance in the sample.

2. There is no single protocol for any isotope tracing or flux experiments (i.e. specific isotope to use, media to use, time points to consider). These experiments are done on a case-by-case basis and depend entirely on the biological question of interest. Always consult with us first regarding what you want to learn from the experiment and subsequent analysis and we are happy to work with you to design an experiment and later analysis that best suits your needs.

Intracellular Metabolite Pellet Post-Extraction
(from subconfluent 6 well plate of adherent cells)



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

Prepared by Locasale Research Group (September 2016)