Guidelines for All Samples-to insure consistency in sample handling:

Materials collected for other experimental work and stored at -80°C can be used for metabolomic studies as long as all of the samples were treated in a consistent way during the collection process, with the following caveats:

Whole tissue Samples:

Whole tissue samples MUST be flash frozen at time of harvest. The preferred way we recommend is to freeze tissue in clamps having ~1“ square metal plates, cooled in liquid nitrogen, or to take tissue, place quickly in a folded sheet of aluminum foil, and smash flat between 2 blocks of dry ice. Then wrap the frozen tissue in the foil, and freeze in liquid nitrogen.

Blood Samples:

1. **Plasma-Human:** For human or other large-volume collection, collect whole blood in tubes (e.g. Vacutainer or Vacuette) containing EDTA (K2, K3 or Na) as anti-coagulant. It is necessary to mix the tube well after collection in the vacutainer, prior to centrifugation. After mixing, centrifuge the samples at ~ 1,000 x g for 10-15 minutes. The clear, upper layer is plasma. Flash freeze in polypropylene tube at -80 deg C.
2. **Plasma-small animal.** For small volume collection, such as small animal tail bleeds, keep the tube cold and quickly determine the volume of blood, then add an equal volume of 9mM EDTA in PBS (to achieve a final concentration of EDTA of approximately 4.5 mM). Mix well and keep cold until centrifugation. As a general guideline, 1.8 ml of whole blood will yield ~1 mL of plasma. For larger blood draws, such as exanguination, we recommend Sarstedt microvette K3EDTA Ref # 20.1341.100 (preferred), or Sarstedt microvette heparin Ref # 20.1345.100) . Flash freeze supernatant in polypropylene tube at -80 deg C.

Plasma Anticoagulant Guidelines:

- Absolutely avoid including multiple anticoagulant sample types in the same experiment (e.g. EDTA plasma, heparin plasma, serum).

1) Serum: Collect whole blood in serum separator tubes and follow tube manufacturer’s processing instructions.

2) Whole blood: Whole blood must be prevented from coagulating, preferably by the addition of EDTA. See the plasma preparation guidelines above, but freeze the sample immediately at -80 deg C after adding the anti-coagulant and mixing well.

3) Volume: 100-200µl is preferable with 100µl a minimum for GC/MS profiling, and at least 50µl for LC/MS profiling. It is also preferable to have consistent

sample amounts for all samples.

• Please notify us of any preservatives that have been used in the collection process.

• Please use polypropylene tubes for sending plasma/blood samples (recommended eppendorf safe-lock 2.0 ml tubes, cat no. 022363344, or Sarstedt tubes with O-ring seal, 2 mL (Sarstedt Cat # 72.694.006 or Fisher Cat # 50809242))

• Please use permanent markers (e.g. Sharpie) to directly label tubes while warm; cold tubes will not hold the ink. Also, stick-on labels tend to fall off when frozen, so unless specialized freezer-safe labels are available, direct marking on the aluminum foil (done prior to experiment) is preferred.

Sample Collection for Metabolites Analysis from mammalian Cell Cultures (100mm-diameter plates)

Samples of ~100mg of wet weight for a cell pellet is easy to process by GC/MS or LC/MS

Make sure to work on ice all the time and as fast it is possible!!!

1. Aspirate off the medium from the cells
2. Wash with 10ml of ice-cold PBS
3. Aspirate off the PBS rapidly
4. Add 10ml of fresh ice-cold PBS
5. Scrape the cells from the plate with a cell lifter (do this rapidly but gentle)
6. Transfer the cell suspension to 15 mL polypropylene tubes (BD-Falcon Cat # 352097 or Fisher Cat # 14-959-70C) or, if a larger volume is needed, a 50 mL polypropylene Falcon Tube may be used (Falcon Cat # 352070 or Fisher Cat # 14-432-22).
7. Spin at about 1000 rpm (200g), by 2 min at 4°C, or use other conditions that have been optimized in your laboratory. Avoid spinning conditions that will lyse the cells.
8. Aspirate off the PBS rapidly and completely. Flash-freeze the cell pellet by immersing the bottom of the tube in N2-liquid

9. Alternatively, instead of step 8, especially when one wants to ship lots of tubes, remove and discard all but ~0.75 mL of the supernatant.

10. Gently resuspend cells and transfer to pre-labeled cryovials, 2.0 mL polypropylene. (Fisher Cat # 5011-0020 or Fisher Cat # 03-341-18D/Wheaton Cat # 985734).

11. Spin at about 1000 rpm (200g), by 2 min at 4°C, or use other conditions that have been optimized in your laboratory again, and carefully remove all

supernatant. If possible, use narrow-bore (gel-loading or small volume) micropipette tips to minimize buffer residual and ensure a dry pellet for freezing.

- Samples should be shipped on dry ice. Tubes should be clearly labeled with sample identifiers and include a hard copy of the sample manifest the quote was given for to accompany the shipment. Please exclude any patient identifiers in the case of human samples.

- An electronic document containing sample ID information should have been uploaded through our website as part of obtaining a quote, and permission for shipment.

- Send actual samples to:

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