Sample Preparation Protocols for Acylcarnitines

General guidelines:

- For cell samples, count the cell number. Tissue samples should be weighed.
- If the user intends to use total protein for normalization, please save the pellets after extraction for protein quantitation.
- For plasma, EDTA (K, Na; avoid Li) is the preferred anticoagulant. Do not use citrate for metabolomics studies. It is recommended to use serum instead of plasma when possible.
- No radioactive samples will be accepted.
- Samples containing infectious pathogens cannot be accepted.

Protocol:

Amount of material needed per sample:

- Cultured cells: > 10 ul cell pellet OR > 2 million cells (estimated based on HEK cells, more cells are needed for smaller cells)
- Solid tissue: > 10mg
- serum/plasma: > 20 ul

From adherent cell cultures

- Detach cells from plates with trypsin or other established methods. Avoid cell lysis.
- Spin down and wash cells with cold PBS twice.
- Transfer cells to a 1.5 ml centrifuge tube
- Spin down the cells and remove liquid from the tube as thoroughly as possible
- Store in -80C till sample submission

- Store at -80C till sample submission

From suspension cell cultures

- Spin down and wash cells with cold PBS twice
- transfer cells to a 1.5 ml centrifuge tube
- Spin down the cells and Remove liquid from the tube as thoroughly as possible

- Store at -80C till sample submission

From solid tissue

- Add >500 ul cold 90% methanol to each 50 ul tissue in 1.5 ml centrifuge tube (volume should be adjusted according to size of tissue)
- Homogenize/smash tissue on ice
- Spin at 14,000 rfc for 20 min at 4C
- Transfer the supernatant to a clean 1.5 ml tube
- Lyophilize or speedvac with no heat on (for hand delivered samples this step can be skipped, but make sure samples are on **dry ice** during transportation. **Rapid heating of extract can lead to popping of centrifuge tubes, resulting in sample loss and possible injury.**)
- Store at -80C till sample submission