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Tissue Sample Preparation for Biochemical Analysis

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We use this protocol and it's working

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Abstract

To prepare tissue samples for general metabolomics profiling by GC-MS or LC-MS.

Guidelines

5. Add one sample to the mortar and grind to a homogenized powder with the pestle
6. Use the cooled spatula to transfer to the labeled screw cap tube
7. Return tube to LN2 while processing other samples.
8. Wipe mortar, pestle and spatula with paper towel or a Kim Wipe prior to processing the next sample. “Rinsing” tools in LN2 can also help remove residual sample.
9. Store tissues at -80$^{°C}$ if not immediately weighing.

Materials

Tissue Collection:
1. Pre-labeled foil squares (3”)
2. Dissection tools
3. Isoflurane vaporizer
4. Liquid nitrogen
5. Aluminum block and tongs

Tissue Pulverization and Weighing:
1. Liquid nitrogen
2. Dry ice
3. Mortar and pestle
4. Spatula
5. Tweezers
6. Ceramic bead ruptor tubes
7. Screw cap tubes

Collection Protocol

1 Fill a cold safe container with liquid nitrogen (LN2) and submerge the flat plates (grip) of the aluminum tongs. Be careful not to allow the tong handles to be submerged. It may be necessary to have extra LN2 on hand to refill the container as it evaporates. If an aluminum block will be used for sample processing, place this on wet ice to cool.

2 Sedate the animal using isoflurane vaporizer.

3 Collect tissues by carefully removing, immediately folding into the pre-labeled foil square, smashing between the cold aluminum tongs, and dropping into LN2. Once samples are snap frozen, they should *always* be kept at -80°C. (If the tissue collection tongs are not available, drop sample folded into foil square directly into LN2.)

Pulverization Protocol

4 Label a set of screw cap tubes (5ml tubes are usually needed for full liver collection from mice; for smaller tissue samples, 2ml tubes are usually sufficient)

5 Float samples (still wrapped in foil) in LN2 during pulverization process. Fill a second LN2 safe container with LN2

6 Cool mortar, pestle, and spatula on dry ice prior to starting pulverization and perform pulverization on dry ice

7 Briefly dip the mortar and pestle into the other container of LN2 to further cool them prior to pulverizing each sample. Leaving some LN2 to evaporate from the mortar during pulverization will help keep tissue cold. Pulverized tissue thaws immediately when warmed; if the sample seems to be “sticky” this means it is beginning to thaw.

8 Add one sample to the mortar and grind to a homogenized powder with the pestle

9 Use the cooled spatula to transfer to the labeled screw cap tube

10 Return tube to LN2 while processing other samples.

11 Wipe mortar, pestle and spatula with paper towel or a Kim Wipe prior to processing the next sample. “Rinsing” tools in LN2 can also help remove residual sample.

12 Store tissues at -80°C if not immediately weighing.