

Standard Operating Procedure: Mechanical dissociation of frozen tissue for protein or RNA extraction

****Work in the biosafety cabinet when using human samples***

Before starting, prepare necessary reagents

- Two buckets of dry ice
 - Bucket of liquid N₂ with spout
 - Mortar and pestle – autoclaved
 - Sterile petri dishes
 - Aluminum foil
 - Small spatula to collect tissue
 - Forceps to remove tissue from tube
 - One sided razor blade
 - 10% bleach solution
 - 70% ethanol
1. Setting up work station in bucket #1 on dry ice:
 - a. Put a sheet of aluminum foil on a bed of dry ice
 - b. Create a “shield” of aluminum foil around the mortar
 - i. Pre-chill instruments and mortar and pestle on foil on dry ice
 2. Pre-weigh and label tubes and record measurements/chill on dry ice
 3. Use your empty dry-ice bucket to obtain samples from -80C
 - a. Can do up to 4 at a time
 4. Pour liquid N₂ into mortar
 5. Using forceps and razor blade:
 - a. Remove sample from tube and place on petri dish on aluminum foil on dry ice
 - b. Using razor blade divide sample as needed (optimal for protein 25-100mg).
 - c. Return remaining sample to tube on dry ice
 - d. Cut off any excess OCT. Place tissue in mortar containing LN₂.
 6. Homogenizing sample:
 - a. Pour additional liquid N₂ into mortar
 - b. Raise aluminum “shield”
 - c. Use gloved hand to act as a barrier and crush tissue with pestle
 - i. Takes more force than you think!
 - d. Continue to add liquid N₂ into mortar and grind as needed until fine powder
 7. Put powder into pre-chilled labeled tube corresponding to sample name
 - a. Add LN₂ and scrape the sidewall of the mortar with spatula to recover the sample
 - b. Do your best to keep tissue frozen at all times (it should not be allowed to be viscous)
 8. Clean utensils with water and 70% ethanol between samples and use a new mortar/pestle
 9. Once done:
 - a. Weigh and record each tube, subtract off weight of empty tube, record tissue weight
 - b. Put all tubes put back in -80C
 - c. Soak mortar/pestle with 10% bleach and then rinse in sink
 - d. Spray down mortar/pestle with 70% ethanol, dry with paper towel, and autoclave
 10. The pulverized tissue can be used for protein and/or RNA analysis following the relevant protocol.