Protocol for Adipose Tissue Homogenization in the Bullet Blender®

The protocol described in this document is for the use of the Bullet Blender® for the homogenization of fat / adipose tissue (from a variety of animals). Note that the time and speed settings may differ due to the variation in consistency/texture of fatty tissue from species to species. This protocol does not specify a particular buffer - you may choose which is most appropriate for your downstream application (nucleic acid isolation, protein extraction, etc.).

Materials Required: adipose tissue, Bullet Blender®, homogenization buffer, pipettor, microcentrifuge tubes and Red bead lysis kit/Pink bead lysis kit/0.5 mm zirconium oxide beads (part number ZrOB05).

Instructions

1. Cut adipose tissue into appropriately sized pieces for analysis (10mg-300mg).
2. **OPTIONAL:** Wash tissue with ~1mL PBS. Aspirate. **NOTE:** This step removes external contaminants (blood, etc.).
3. a. *Samples 100mg or greater*
   Place the sample in Red bead lysis kit tube.
   b. *Samples less than 100mg*
   Place the sample in Pink bead lysis kit tube.
   c. *Alternate protocol step for bulk beads*
   Place sample in microcentrifuge tube and add beads to the tube. Use a volume of beads equal to the mass of tissue. **NOTE:** 100mg ≅ 100µL.
4. Add 0.025mL to 0.6mL buffer (2 volumes of buffer for every mass of tissue).
5. Close the microcentrifuge tubes.
6. Place tubes into the Bullet Blender®.
7. Set controls for **SPEED 8** and **TIME 3** minutes. Press **Start**.
8. After the run, remove tubes from the instrument.
9. Inspect samples. Fatty tissue homogenate will be difficult to see through due to the light scattering of lipid micelles formed, so it may be necessary to employ a pipette tip to check inside the tube for remaining pieces of intact tissue. If homogenization is unsatisfactory, run for another minute at **SPEED 9**.
10. Proceed with your downstream application.

SAFETY NOTE!!! – Make sure your tubes are balanced before placing them into a centrifuge!

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