Protocol for Testes Tissue Homogenization in the Bullet Blender®

The protocol described in this document is for the use of the Bullet Blender® for the homogenization of testes tissue (from a variety of animals). Note that the time and speed settings may differ due to the variation in consistency/texture of 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: testes tissue, Bullet Blender®, homogenization buffer, pipettor, microcentrifuge tubes, and Navy bead lysis kit/Pink bead lysis kit/0.5mm zirconium oxide beads (part number ZSB05) or 0.9-2.0mm stainless steel beads (part number SSB14B).

Instructions

1. Cut testes tissue into appropriately sized pieces for analysis (10mg-300mg).
2. **OPTIONAL:** Wash tissue 3x with ~1mL PBS. Aspirate. **NOTE:** This step removes external contaminants (blood, etc.).
3. a. *Samples 100mg or greater*
   Place the sample in Navy 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 (either zirconium oxide, stainless steel, or a combination). 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 volume of sample).
5. Close the microcentrifuge tubes tightly and place them into the Bullet Blender®.
6. Set controls for **SPEED 9** and **TIME 3** minutes. Press **Start**.
7. After the run, remove tubes from the instrument.
8. Visually inspect samples. If homogenization is unsatisfactory, run for another two minutes at the **SPEED 10.**
9. Proceed with your downstream application.

SAFETY NOTE!!!

When using a centrifuge to separate your homogenate from the debris and beads, make sure your tubes are balanced.

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