Protocol for Intestinal Tissue Homogenization in the Bullet Blender®

The protocol described in this document is for the use of the Bullet Blender® for the homogenization of intestinal tissue. Note that the time and speed settings, and digestion parameters 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: intestine tissue, Bullet Blender®,homogenization buffer, microcentrifuge tubes, pipettor, and Navy bead lysis kit/Green bead lysis kit/0.9-2.0mm stainless steel bead blend (product number SSB14B).

Instructions

1. Cut intestinal tissue into appropriately sized pieces for analysis (10-300mg).
2. **OPTIONAL:** Wash tissue 3x with ~1mL PBS. Aspirate. **NOTE:** This step removes any external contaminants (blood, undigested food, etc.).
3. a. *Samples 50mg or greater*
   Place the sample in Navy bead lysis kit tube.
   b. *Samples less than 50mg*
   Place the sample in Green 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 0.025 mL to 0.6mL buffer (2 volumes of buffer for every volume of sample).
5. Close the centrifuge tubes.
6. Place tubes into the Bullet Blender.
7. Set controls for **SPEED 8** and **TIME 4** minutes. Press **Start**.
8. After the run, remove tubes from the instrument.
9. Visually inspect samples. If homogenization is unsatisfactory, run for another two minutes at the **SPEED 10.**
10. 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.

Date 05/06/2011