Protocol for Jejunum or Stomach Tissue Homogenization in the Bullet Blender®

The protocol described in this document is for the use of the Bullet Blender® for the homogenization of jejunum or stomach / gastric tissue. 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: stomach 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) and 3.2mm stainless steel balls.

Instructions

1. Cut tissue into appropriately sized pieces for analysis (10-300mg). **NOTE:** Try to remove pieces of connective tissue as they do not homogenize well.

- 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 head lysis kit to
 - Place the sample in Navy bead lysis kit tube. b. *Samples less than 50mg*
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 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 0.9-2.0 stainless steel bead blend equal to the mass of tissue. Optionally, add 1-5 3.2mm stainless steel balls. **NOTE:** $100 \text{mg} \approx 100 \mu \text{L}$.
- **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.