Protocol for Uterine Tissue Homogenization in the Bullet Blender[®]

The protocol described in this document is for the use of the Bullet Blender[®] for the homogenization of uterine tissue (from a variety of animals). Note that the time and speed settings may differ due to the variation in consistency/texture of different 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:

uterine tissue, Bullet Blender[®], microcentrifuge tubes, Navy bead lysis kit/Green bead lysis kit/stainless steel beads (1.6mm, product number SSB16 or 0.9-2.0mm blend, product number SSB14B), homogenization buffer, and pipettor.

Instructions

- 1. Cut uterine tissue into appropriately sized pieces for analysis (10mg-300mg).
- **2. OPTIONAL:** Wash tissue 3x with ~1mL PBS. Aspirate. **NOTE:** This step removes some external contaminants (blood, 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:** 100μ L.
- **4.** Add 0.025mL to 0.6mL buffer (2 volumes of buffer for every volume of sample).
- **5.** Close the microcentrifuge tubes.
- **6.** Place tubes into the Bullet Blender[®].
- 7. Set controls for SPEED 8 and TIME 5 minutes. Press Start.
- **8.** After the run, remove tubes from the instrument.
- **9.** Visually inspect samples. If homogenization is unsatisfactory, run for another three minutes at the **SPEED 10.**
- **10.** Remove sample tubes from the Bullet Blender[®], add the appropriate buffer and 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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Quasar Instruments, LLC 4835 Centennial Blvd. Colorado Springs, CO 80919

after

T. 888-535-8001

D. 719-535-8000

F. 719-574-2404

E. Sales@qi-us.com