Date 11/23/2009

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.).

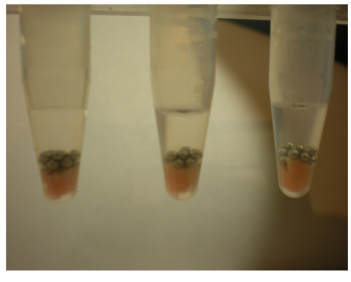
Materials Required: uterine tissue, saline, aspirator, Bullet Blender™, homogenization buffer, pipettor, microcentrifuge tubes, 0.9 to 2.0 mm stainless steel bead blend (part number SSB14B) or 1.6 mm stainless steel beads (part number SSB16).

Instructions

1. Cut uterine tissue into appropriately sized pieces for analysis (50mg-150mg, if your tissue is not already smaller) and place into microcentrifuge tubes.
2. **OPTIONAL:** Wash tissue 3x with ~1mL PBS. Aspirate. **NOTE:** This step removes some external contaminants (blood, etc.).
3. Add a mass of the stainless steel beads equal to 3X the mass of the sample. One scoop of stainless steel blend ≈ 220mg. One scoop of 1.6 mm stainless steel beads ≈ 186mg.
4. Add 2 volumes of buffer for every mass of tissue, minimum of 25µL.
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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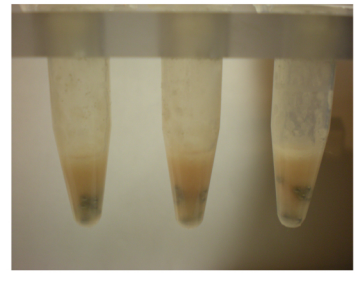

after

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