Protocol: Umbilical Cord tissue Homogenization in the Bullet Blender®

The protocol described in this document is for the use of the Bullet Blender® for the homogenization of umbilical cord 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: umbilical cord tissue, Bullet Blender®, homogenization buffer, pipettor, microcentrifuge tubes, and Navy bead lysis kit/Green bead lysis kit/0.9-2.0mm stainless steel bead blend (product number SSB14B).

Instructions

1. Cut umbilical cord 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 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 0.025mL to 0.6mL buffer (2 volumes of buffer for every mass of tissue).
5. Close the microcentrifuge tubes tightly, and place the tubes into the Bullet Blender®.
6. Set controls for **SPEED 10** and **TIME 5** minutes. Press start.
7. Remove tubes from the instrument.
8. Visually inspect samples, if homogenization is unsatisfactory, run for another three minutes at **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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