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Protocol for Epithelial Tissue Homogenization in the Bullet Blender™

The protocol described in this document is for the use of the Bullet Blender™ for the homogenization of epithelial tissue (from a variety of animals). Note that the time and speed settings may differ due to the variation in consistency/texture of various types of epithelial tissue, and variations 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: epithelial tissue, saline, Bullet Blender™, homogenization buffer, pipettor, microcentrifuge tubes, and 0.5mm zirconium silicate beads (part number ZSB05).

Instructions

1. Cut tissue into appropriately sized pieces for analysis (5mg-300mg) and place into a microcentrifuge tube. If possible, use long thin tissue pieces.
2. **OPTIONAL:** Wash tissue 3x with ~1mL PBS. Aspirate. **NOTE:** This step removes external contaminants (blood, etc.).
3. Add zirconium silicate beads (0.5mm) to the tube. Use a mass of beads equal to 1.5x your mass of tissue. One scoop of beads ≈ 192mg.
4. Add 0.025mL to 0.6mL buffer (2 volumes of buffer for every volume of cells, 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 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.

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