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# Protocol for Lung / Tracheal Tissue Homogenization in the Bullet Blender™

The protocol described in this document is for the use of the Bullet Blender™ for the homogenization of pulmonary (lung) or tracheal 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:** pulmonary or tracheal tissue, Bullet Blender™, homogenization buffer, pipettor, microcentrifuge tubes, and 0.9-2.0mm stainless steel bead blend (part number SSB14B).

**Instructions**

1. Cut lung or tracheal tissue tissue into appropriately sized pieces for analysis (50mg-300mg) and place into a microcentrifuge tube. Typical sample size: 200mg – about ¼ of a rat lung.
2. **OPTIONAL:** Wash tissue 3x with ~1mL PBS. Aspirate. **NOTE:** This step removes external contaminants (blood, etc.).
3. Add stainless steel beads (0.9-2.0mm). Use a mass of beads equal to your mass of tissue. **NOTE:** 100mg of beads ≅ 50µL.
4. Add 0.1mL to 0.6mL buffer (2 volumes of buffer for every mass of tissue).
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 8.**
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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