

Protocol for Pulmonary (Lung) 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) 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 tissue, Bullet Blender[®], homogenization buffer, pipettor, microcentrifuge tubes, and Navy bead lysis kit/**Pink bead lysis kit**/0.9-2.0mm stainless steel beads (part number SSB14B).

Instructions

1. Cut lung tissue into appropriately sized pieces for analysis (10mg-300mg) and place into a microcentrifuge tube.
2. **OPTIONAL:** Wash tissue 3x with ~1mL PBS. Aspirate. **NOTE:** This step removes external contaminants (blood, etc.).
3.
 - a. *Samples 100mg or greater*
Place the sample in Navy bead lysis kit tube.
 - b. *Samples less than 100mg*
Place the sample in **Pink bead lysis kit** tube.
 - c. *Alternate protocol step for bulk beads*
Place sample in microcentrifuge tube and add beads to the tube (either zirconium oxide, stainless steel, or a combination). Use a volume of beads equal to the mass of tissue. **NOTE:** 100mg \cong 100 μ L.
4. Add 0.025mL 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.