

Protocol for Eye Tissue Homogenization in the Bullet Blender®

The protocol described in this document is for the use of the Bullet Blender® for the homogenization of eye tissue (from a variety of animals). The homogenization time and speed settings may differ due to the variation in consistency/texture of tissue from species to species. **Note: corneal and lens tissue may require enzymatic pretreatment with collagenase and / or hyaluronidase in order to achieve good homogenization.** 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: optic tissue, Bullet Blender®, homogenization buffer, pipettor, microcentrifuge tubes and Red bead lysis kit/Pink bead lysis kit/0.5 mm zirconium oxide beads (part number ZROB05).

Instructions

1. Cut eye tissue 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 100mg or greater*
Place the sample in Red 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. Use a volume of beads equal to the mass of tissue. **NOTE:** 100mg \approx 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 3** minutes. Press **Start**.
8. After the run, remove the tubes from the instrument.
9. Visually inspect samples. If homogenization is unsatisfactory, run for another two minutes at the **SPEED 9**.
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!