

Bullet Blender® 50

Homogenization Protocol for Eye Tissue

The protocol described in this document is for the use of the Bullet Blender® 50 for the homogenization of Eye Tissue. **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:

eye tissue , Bullet Blender® 50, homogenization buffer, pipettor, 50mL skirted centrifuge tubes (Axygen® or Corning® brand), 4.8mm stainless steel beads (part number SSB48) or 3.2mm stainless steel beads (part number SSB32).

Instructions

1. Cut eye tissue into appropriately sized pieces for analysis (0.1g – 3.5g) and place into a 50mL centrifuge tube.
2. **OPTIONAL:** If desired, wash the tissue 3x with 5mL PBS to remove blood and other contaminants from the tissue.
3. Add a mass of stainless steel beads to the tube equal to approximately 6x the mass of your sample.
4. Add 0.2 mL to 7mL buffer (2 volumes of buffer for every mass of sample).
5. Screw caps onto centrifuge tubes **TIGHTLY**.
6. Place tubes into the Bullet Blender® 50.
7. Set controls for **SPEED 8** and **TIME 12** minutes.
8. Remove tubes from the instrument.
9. Visually inspect samples, if homogenization is unsatisfactory, run for another six 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.

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