NEXTADVANCE

Protocol for Mouse Femur Homogenization in the Bullet Blender®

The protocol described in this document is for the use of the Bullet Blender® for the homogenization of mouse femur or other small brittle bone tissue. 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.).

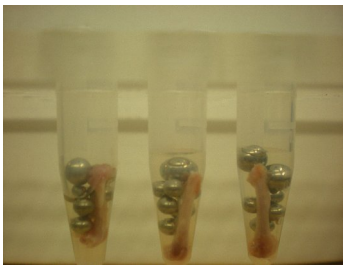
Materials Required: Femur, Bullet Blender®,homogenization buffer, microcentrifuge tubes, pipettor, and Navy bead lysis kit/0.9-2.0mm stainless steel bead blend (product number SSB14B) and 3.2mm stainless steel balls (SSB32)*.

Instructions

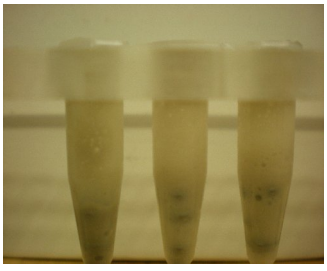
1. Choose appropriately sized pieces for analysis (10-100mg).
2. OPTIONAL: Wash tissue 3x with ~1mL PBS. Aspirate. NOTE: This step removes any external contaminants (blood etc.).
3. a. P*rotocol step for pre-loaded tubes*
   Place the sample in Navy bead lysis kit tube.
   c. P*rotocol step for bulk beads*
   Place sample in microcentrifuge tube and add ~100µL of the stainless steel blend and 6 x 3.2mm stainless steel balls.
4. Add 0.2 mL to 0.6mL buffer (~2 volumes of buffer for every volume of beads).
5. Close the centrifuge tubes.
6. Place tubes into the Bullet Blender.
7. Set controls for SPEED 10 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 5 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.



Before



After

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QUASAR INSTRUMENTS
QuasarInstruments.com

Quasar Instruments, LLC
4835 Centennial Blvd.
Colorado Springs, CO 80919

T. 888-535-8001 D. 719-535-8000 F. 719-574-2404 E. Sales@qi-us.com