Protocol for Tumor Homogenization in the Bullet Blender®

The protocol described in this document is for the use of the Bullet Blender® for the homogenization of tumor / cancer tissue (from a variety of animals). This protocol was developed using carcinoma. Note that due to the highly varied nature of tumors, especially tumors arising from different tissues, you may need to modify this protocol to suit your specific needs. 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: tumor, tissue, Bullet Blender®,homogenization buffer, microcentrifuge tubes, pipettor, and Navy bead lysis kit/Green bead lysis kit/1.6 mm stainless steel beads (product number SSB16).

Instructions

1. Cut tumor tissue into appropriately sized pieces for analysis (10-300mg).
2. OPTIONAL: Wash tissue 3x with ~1mL PBS. Aspirate. NOTE: This step removes external contaminants (blood, etc.).
3. a. *Samples 50mg or greater*
   Place the sample in Navy bead lysis kit tube.
   b. *Samples less than 50mg*
   Place the sample in Green 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 ≅ 100µL.
4. Add 0.025 mL to 0.6mL buffer (2 volumes of buffer for every volume of sample).
5. Close the microcentrifuge 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. Inspect samples. If homogenization is unsatisfactory, run for another five minutes at SPEED 10.
10. Proceed with your downstream application.

SAFETY NOTE!!! – Make sure your tubes are balanced before placing them into a centrifuge!

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