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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, buffer, Bullet Blender™, microcentrifuge tubes, 1.6mm stainless steel beads (part number SSB16), and pipettor.

Instructions

1. Cut tumor tissue into appropriately sized pieces for analysis (250mg or less) and place into a microcentrifuge tube.
2. **OPTIONAL:** Wash tissue 3x with ~1mL PBS. Aspirate. **NOTE:** This step removes external contaminants (blood, etc.).
3. Add a mass of 1.6mm stainless steel beads equal to 1.3 times your mass of tissue (so if you had 100mg of tissue, you would use 130mg of beads). One bead weighs 17mg.
4. Add 3 volumes of buffer for every mass of tissue (so for 100mg of tissue, you would add 300ml of buffer).
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!

Reference:

Schewe, D.M, Aguirre-Ghiso, J.A., ATF6alpha-Rheb-mTOR signaling promotes survival of dormant tumor cells in vivo. Proc Natl Acad Sci USA, 2008 Jul 29;105(30):10519-24

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