Homogenization in the Bullet Blender® 50 Protocol for Mammalian Cell Culture

The protocol described in this document is for the use of the Bullet Blender® 50 for the homogenization of mammalian cell cultures. 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: mammalian cells, Bullet Blender® 50, homogenization buffer, pipettor, 50mL skirted centrifuge tubes (Axygen® or Corning® brand), 0.15mm zirconium oxide beads (part number ZROB015) or 0.1mm glass beads (part number GB01).

Instructions

1. Detach cells from culture dish or flask by your chosen method (trypsinization, scraping, spontaneous detachment, etc.)
2. Wash cells from dish with PBS into 50ml tube
3. Centrifuge cell suspension to yield a cell pellet (200-500g for five minutes at 0°C).
4. Completely aspirate the supernatant liquid. Place tube on ice.
5. Inspect the volume of the pellet. It should be 4mL or less to achieve efficient homogenization.
6. Completely aspirate supernatant liquid. Place tube on ice.
7. Add a volume of beads to the tube approximately equal to the volume of the pellet.
8. Add 0.2 mL to 8mL buffer (2 volumes of buffer for every volume of sample).
9. Screw caps onto centrifuge tubes **TIGHTLY**.
10. Place tubes into the Bullet Blender® 50.
11. Set controls for **SPEED 8** and **TIME 12** minutes.
12. Remove tubes from the instrument.
13. Visually inspect samples, if homogenization is unsatisfactory, run for another six minutes at the **SPEED 9**.
14. 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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