Protocol for Use of the Bullet Blender™ in Tandem with the QuickGene-Mini80-Cultured Cells

The protocol described in this document is for the use of the Bullet Blender™ in tandem with the Fuji QuickGene-Mini80 RNA isolation system for cultured mammalian cells. Cells will first be homogenized using the Bullet Blender™ with the buffers provided in the QuickGene kit and biological sample provided by the researcher.

Materials Required: Cultured Cells, Bullet Blender™, QuickGene kit, pipetor, microcentrifuge tubes, and 0.15mm zirconium oxide beads (part number ZrOB015-RNA) or 0.1mm glass beads (GB01-RNA)

Instructions

1. Detach cells from culture dish or flask by your chosen method (trypsinization, scraping, spontaneous detachment, etc.).
2. Transport the appropriate number of cells according to the QuickGene protocol into a microcentrifuge tube.
3. Centrifuge cell suspension for 5 min at 300Xg to yield a cell pellet.
4. Completely aspirate the supernatant liquid. Loosen pellet by flicking tube. Place tube on ice.
5. Inspect the volume of the pellet. It should be 300µL or less.
6. Add a volume of zirconium oxide beads (0.15mm) **OR** glass beads (0.1mm) to the tube equal to the volume of the pellet. One scoop of beads ≈ 50µL.
7. Add 2 volumes of LRP buffer (from QuickGene kit, with 2-mercaptoethanol) for every volume of cells.
8. Close the microcentrifuge tubes.
9. Place tubes into the Bullet Blender™.
10. Set controls for **SPEED 5** and **TIME 2 to 3** minutes. Press **Start**.
11. After the run, remove tubes from the instrument. Visually inspect samples. If homogenization is unsatisfactory, run for another two minutes at **SPEED 5.**
12. Prior to removing samples from the Bullet Blender, open tube cap, then add LRP to reach 350 µl if you are using QuickGene Protocol A, 600 µl if you are using QuickGene Protocol B, or 800 µl if you are using QuickGene Protocol B'. Close cap. Run Bullet Blender at **SPEED 2** for **1 minute** to thoroughly mix.
13. Proceed with QuickGene protocol (i.e. SRP addition and flash spin).

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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