Protocol for Use of the Bullet Blender® in Tandem with the QuickGene-Mini80

The protocol described in this document is for the use of the Bullet Blender® in tandem with the Fuji QuickGene-Mini80 RNA isolation system. Tissue 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: tissue, Bullet Blender®, QuickGene kit, pipetor, microcentrifuge tubes, and 0.5mm zirconium oxide beads (part number ZrOB05)

Instructions

1. Place tissue into a 1.7mL microcentrifuge tube.
2. Add the appropriate mass zirconium oxide beads (0.5mm). Measurement by mass or volume is equally valid, you may find it more expedient to use the scoop provided by NEXT›››ADVANCE (see table below).
3. Add appropriate volume of LRT buffer (with 2-mercaptoethanol, in QuickGene kit).

Tissue mass	*Bead mass*	*Bead volume*	*LRT volume*
5-15mg	50mg	1 scoop	100µL
15-30mg	100mg	2 scoops	200µL

4. Close microcentrifuge tubes.
5. Place tubes into the Bullet Blender®, and run at a speed appropriate for the tissue type as specified in this table.

Tissue type	*Speed*	*Time (min)*
brain	6	3
liver	8	2
skin	8	3
spleen	8	3
kidney	8	4
heart	10	5

6. Visually inspect samples. If homogenization is unsatisfactory, return tubes to the Bullet Blender®, run for another two minutes at the speed specified for the sample type.
7. Prior to removing samples from the Bullet Blender, open tube cap, then add LRT to reach the volume specified in the QuickGene protocol (300µL or 400µL, respectively). Close cap. Run Bullet Blender at **SPEED 2** for **1 minute** to thoroughly mix.
8. Proceed with QuickGene protocol (i.e. centrifugation at room temperature).

SAFETY NOTE!!!

When using a centrifuge to separate your homogenate from the debris and beads, make sure your tubes are balanced.

Date 05/06/2011