Mammalian Cell Culture Homogenization Using the Bullet Blender

Materials

RS18-0238B.5MCC

- <u>Bullet Blender</u>[®] for 5 mL tubes
- Homogenization Buffer
- <u>FoamBlocker</u> (Optional)
- Lysis Kit or Lysis Beads
 - PINK or RED Lysis Kit (from <u>PrecisionPak™</u> or purchased separately)
 - 0.15 mm Zirconium Oxide Beads in Eppendorf or GATOR tubes
- Sample up to 1000 mg

Table 1. Proper sample, bead and buffer volume ratios for 5 mL tubes.

Lysis Kit and Bead Choices	Sample Volume	Bead Volume	Buffer Volume
PINK	300 μL pellet	Pre-filled	0.5 - 1.2 mL
RED	1000 µL pellet	Pre-filled	1.2 - 2.5 mL
0.15 mm Zirconium Oxide Beads	up to 1000 µL pellet	500 - 800 μL	1.2 - 2.5 mL

Procedure

- 1. Use the pre-filled bead lysis kit tubes OR prepare a tube with the recommended volume of bead choices from the table above.
- 2. If the sample has been grown on a plate or other solid surface, detach it by flooding the surface with PBS and scraping the material into a microcentrifuge tube. Centrifuge to obtain a cell pellet and resuspend in the specified volume of lysis buffer. Liquid cultures may be placed directly in the lysis tube as long as they are of sufficient density.
- 3. (Optional) To avoid excess foaming, add FoamBlocker up to 1-2% of the total volume of the homogenization buffer.
- 4. Close the tubes tightly and place into the Bullet Blender sample chamber. If using the Gold or Gold⁺ models, pre-cool the chamber before adding sample tubes.
- 5. Set the controls to speed 8, time 2 minutes then press Start. *Note: Using single-size beads instead of pre-filled lysis kits may require additional time.*
- 6. After the run, remove the tubes from the instrument and visually inspect the samples. If homogenization is incomplete, homogenize for an additional 30 seconds, or repeat the homogenization step with a higher speed.
- 7. Using a pipette, transfer the homogenized samples into new tubes.
- 8. Proceed with downstream application.

Notes

This protocol does not specify a particular buffer – choose a buffer that is most appropriate for the downstream application or use the lysis buffer provided in a <u>PrecisionPak</u>TM, a simplified workflow solution which also includes a bead lysis kit, supplemental reagents for high quality nucleic acids isolations, and an optimized protocol for specific samples.

(development_note)



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