E. coli Homogenization Using the Bullet Blender

RS18-0238B.5ECO

Materials

- Bullet Blender® for 5 mL tubes
- Homogenization Buffer
- FoamBlocker (Optional)
- Lysis Beads
    - 0.15 mm Zirconium Oxide Beads in Eppendorf or GATOR tubes
- Sample — up to 1000 µL pellet

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

Bead Choices	Sample Volume	Bead Volume	Buffer Volume
0.15 mm Zirconium Oxide Beads	up to 1000 µL pellet	500 - 800 µL	1.2 - 2.5 mL

Procedure

1. 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 3 minutes then press Start.
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 PrecisionPak™, 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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