Alfalfa Seed Homogenization Using the Bullet Blender

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Materials

- Bullet Blender® for 1.5/2 mL tubes using 5 mL adapters
- Homogenization Buffer
- FoamBlocker (Optional)
- Lysis Kit or Lysis Beads
 - GREEN or NAVY Lysis Kit (from <u>PrecisionPak™</u> or purchased separately)
 - 3.2 mm Stainless Steel Beads in Eppendorf, GATOR, or RINO tubes
- Sample up to 200 mg (no reagents required)

Table 1. Proper sample, bead and buffer volume ratios for 1.5/2 mL tubes using 5 mL adapters.

Lysis Kit and Bead Choices	Sample Volume	Bead Volume	Buffer Volume
GREEN	Up to 100 mg	Pre-filled	200 - 300 μL
NAVY	100 - 200 mg	Pre-filled	300 - 600 μL
3.2 mm Stainless Steel Beads	Up to 200 mg	6 beads	200 - 600 μL

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. Add the appropriate volume of buffer according to the table above
- 3. Prepare the sample by crushing it into small thin pieces and then transfer it into the buffer-filled tubes.
- 4. (Optional) To avoid excess foaming, add FoamBlocker up to 1-2% of the total volume of the homogenization buffer.
- 5. 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.
- 6. Set the controls to speed 12, time 5 minutes then press Start.

 Note: Using single-size beads instead of pre-filled lysis kits may require additional time.
- 7. 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.
- 8. Using a pipette, transfer the homogenized samples into new tubes.
- 9. 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>, 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.

The provided homogenization conditions serve as a general guideline. Homogenization times, speeds, or beads may need to be optimized based on sample characteristics and desired outcomes.

