Protocol for A. thaliana Homogenization in the Bullet Blender®

The protocol described in this document is for the use of the Bullet Blender® for the homogenization of Arabidopsis thaliana tissue (leaves, stems, seeds, or roots). This protocol does not specify a particular buffer - you may choose which is most appropriate for your downstream application (nucleic acid isolation, protein extraction, etc.).

Materials Required: plant material, Bullet Blender®, homogenization buffer, pipettor, microcentrifuge tubes, and Red bead lysis kit /0.5mm zirconium oxide beads (part number ZrOB05).

Instructions

1. Try to remove as much dirt or other debris from the plant by gently shaking.
2. If desired, wash the with saline/distilled water to remove any soil or other particulate matter that is not part of the plant.
3. Blot excess liquid from the soft tissue using a Kimwipe® or other lint free cloth.
4. Cut the plant material into appropriately sized pieces (up to approximately 300µl volume of plant per tube).
5. a. Place the plant tissue into a Red bead lysis kit microcentrifuge tube.
   b. Alternate protocol step for bulk beads
   Plave the sample in a microcentrifuge tube and add 0.5mm zirconium oxide beads to the tube. Use a volume of beads equal to the volume of the sample.
6. Add 0.1m to 0.6mL buffer, i.e. twice as much buffer as sample.
7. Close the microcentrifuge tubes.
8. Place tubes into the Bullet Blender®.
9. Set controls for SPEED 8 and TIME 2 to 3 minutes. Press Start.
10. After the run, remove tubes from the instrument.
11. Visually inspect samples. If homogenization is unsatisfactory, run for another five minutes at the SPEED 10.
12. Proceed with your downstream application.

SAFETY NOTE!!!

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

Latest revision: 20 April 2012