

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 [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 tissue into appropriately sized pieces (0.05 to 0.3g).
5. Place the plant tissue into a microcentrifuge tube.
6. Add 0.05g to 0.3g zirconium oxide beads (0.5mm) to the tube. One scoop is approximately 0.1g beads.
7. Add 0.1m to 0.6mL buffer, i.e. twice as much buffer as sample.
8. Close the microcentrifuge tubes.
9. Place tubes into the Bullet Blender™.
10. Set controls for **SPEED 8** and **TIME 2 to 3** minutes. Press **Start**.
11. After the run, remove tubes from the instrument.
12. Visually inspect samples. If homogenization is unsatisfactory, run for another five minutes at the **SPEED 10**.
13. 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.

The text above describes a generic protocol. A specific measure of materials from two different protocols are listed here:

Protocol A

Leaf tissue: ~0.05g

(snap centrifuge cap closed on leaf)

Buffer: 500µL

ZrO beads

SPEED 8, TIME 3 minutes

Protocol B

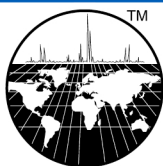
Leaf tissue: ~0.05g

(snap centrifuge cap closed on leaf)

Buffer: 450-500µL

Stainless Steel beads (1.6mm)

SPEED 8, TIME 2 to 3 minutes



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