NEXTADVANCE

Protocol for Pine Nut Homogenization in the Bullet Blender®

The protocol described in this document is for the use of the Bullet Blender® for the homogenization of pine nut (seeds from the genuus *Pinus*). Note that the time and speed settings may differ due to the variation in consistency/texture of different species. 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.).

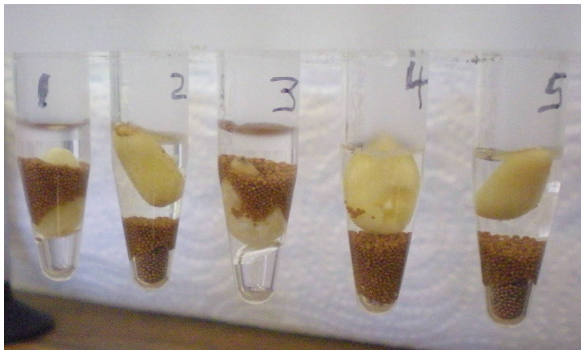
Materials Required: pine nuts, saline, aspirator, Bullet Blender®, homogenization buffer, pipettor, microcentrifuge tubes, 0.5mm zirconium oxide beads (part number ZrOB05)

Instructions

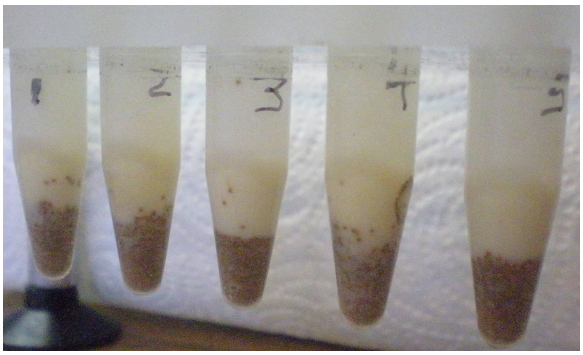
1. Place one nut (100-200mg) into a microcentrifuge tube.
2. **OPTIONAL:** Wash nut 3x with ~1mL PBS. Aspirate. **NOTE:** This step removes some external contaminants and debris.
3. Add 0.6g of zirconium oxide beads (0.5mm) to the tube.
4. Add 0.4mL buffer to the tube.
5. Close the microcentrifuge tubes.
6. Place tubes into the Bullet Blender®.
7. Set controls for **SPEED 10** and **TIME 4** minutes. Press **Start**.
8. After the run, remove tubes from the instrument.
9. Visual inspection of the homogenate is difficult due to the formation of emulsions with the oils from the plant tissue in aqueous buffers. To verify sufficient homogenization, you may place forceps or a spatula inside the tube to search for remaining large fragments. If homogenization is unsatisfactory, run for another two minutes at the **SPEED 10.**
10. Remove sample tubes from the Bullet Blender® and 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.



before



after

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