

MAKING RNA WITH THE MINIBEADBEATER-8

Purpose: To extract RNA from samples of interest.

Materials Needed:

- 0.1mm glass beads (for bacteria) (Biospec products, cat# 11079101)
- 0.5mm glass beads (for yeast, fungi, and tissue culture cells) (Biospec products, cat# 11079105)
- 1.0mm zirconia/silica beads (for plant and animal tissue) (Biospec products, cat# 11079110z)
- 2ml screw-cap microtubes with o-ring seals (either the tubes from Biospec cat# 522S or another comparable product are suitable)
- Minibeadbeater-8 (Biospec products, cat# 693)

Procedure:

1. Pre-chop solid tissue into approximate 1mm cubes with a razor blade.
2. Fill the 2ml microtube one-half to two-thirds full of beads.
3. Add initial lysis media and cells, being sure to fill the tube almost to the top. (Lysis media may be RNAzol, Trizol, lysis buffer from RNAeasy kit, etc)
4. Exclude as much air from the microtube as possible and screw top on.
5. Insert one to eight microtubes into the chamber holder, distributing symmetrically.
6. Screw in the black plastic chamber cap, flat side down, until it is in contact with the tops of the microtube caps.
7. Screw on tight the white nylon wing nut.
8. Set the toggle switch on the control box to "Time" and select the speed. **A typical setting for cell disruption is 3 minutes at "Homogenize."
9. Start the homogenization by pushing the white button in the middle of the timer dial. The timer dial resets itself automatically at the end of the run.
10. Centrifuge for 10 minutes at 10000 rpm in an eppendorf tube.
11. Remove RNA containing layer (layer will differ depending on the extraction media used).
12. Reseal the microtube and discard (samples containing organic solvents must be disposed of properly).

Comments:

The MiniBeadBeater-8 runs at a high volume which may exceed 85 db. It may be advisable to isolate the machine.

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For frequently asked questions go to the following address:

<http://www.grc.nia.nih.gov/branches/rrb/dna/protocolFAQs.htm>

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