Yeast total RNA isolation protocol with Phenol:Chloroform

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This is a yeast total RNA isolation protocol that works very well and reliably. It takes a couple of hours and must be done in a fume hood. We have also had success with the Zymo columns, Direct-Zol or RNA Clean and Concentrator, which are faster but can be less clean if overloaded. For general advice working with RNA consult RNA: A Laboratory Manual from Cold Spring Harbor Press.

Use RNase-free water, reagents, and equipment throughout.

1. Grow up 5ml liquid yeast culture to OD 2.0 for lots of RNA (or as desired). Spin at 3000g for 4min at room temperature. Remove supernatant and resuspend pellet in 500ul RNA lysis buffer (10mM Tris-HCl pH8.5, 5mM EDTA, 2% SDS, 2% stock 2-mercaptoethanol).
2. Transfer liquid to 1.5ml tube and put on heat block at 83 with 450rpm mixing for 20mins (to disrupt cells and denature proteins).
3. Spin down for 5mins at 12,000g.
4. Take supernatant into new tube and add 550 ul of Phenol pH8. Vortex for 15 mins[1].
5. Spin down for 2mins at 12,000g. There should be a lower phenol phase, a cloudy interphase, and an upper aqueous phase: RNA partitions mostly into the upper phase. Transfer upper aqueous phase (roughly 200 ul) to new 1.5ml tube (labeled tube N). Add 250 ul RNA lysis buffer to previous tube (labeled tube P) and vortex tube P for 5mins.
6. Add 250 ul chloroform to tube P to suck off phenol from water phase (This completes a phenol:chloroform extraction of RNA from the original sample). Vortex for 3 mins, then spin 2mins at 12,000g and transfer aqueous phase from tube P to tube N. Discard tube P.
7. A second phenol extraction: add 550 ul phenol to tube N. Vortex 5mins, spin 2mins at 12,000g.
8. Transfer aqueous phase to another 1.5ml tube, discard tube N. Add 550 ul phenol:chloroform pH4.5. Vortex 3mins, then spin 2mins at 12,000g.
9. Transfer 450 ul aqueous phase to yet another 1.5ml tube, add 200 ul of 0.6M Sodium acetate pH4.5, mix by flicking, and spin briefly. Add 600 ul of phenol:chloroform pH4.5. Vortex 5mins, then spin 2 mins at 12,000g.
10. Transfer 350 ul aqueous phase to a fresh 1.5ml tube, add 30 ul 5M Ammonium acetate and 1.1ml ethanol. Mix well, and precipitate at -80 for 20mins. The sample may be left for longer, for example overnight, at this point if a pause is desired.
11. Remove the sample from freezer. Cold spin (4) for 15 mins at 12,000g.
12. Thoroughly remove ethanol from pellet, and add 700 ul 80% ethanol. Cold spin (4C) for 2 minutes at 12,000g. Repeat the ethanol wash and cold spin. This removes all traces of salt, SDS, etc.
13. Dry pellets thoroughly, i.e., pipette off ethanol, removing all liquid. If necessary, dry with the tubes open on a 37 heat block (if the RNA sample is pure, this should not degrade the RNA). Resuspend pellet in 50 ul H_2O.
14. To check the quality of the RNA, pour a 1% agarose gel on RNA-free equipment, and run using RNA loading dye supplied with a ladder (RiboRuler High Range, ThermoFisher #SM1821, or ssRNA Ladder, NEB #N0362S). Heat loading dye and H_2O to 95 for 5 minutes, and then cool, to reduce the possibility of contamination. Mix 1 ul sample, 5 ul H_2O, and 6 ul 2X loading dye for each well. You should see clear ~4000nt and ~2000 nt bands corresponding to ribosomal RNA (25S/18S), also a blur at ~80nt for tRNA, and good gels show the 5S and 5.8S rRNAs at ~150nt. Perform a 2X serial dilution of the sample for more precise quantification, or run on Agilent Bioanalyzer RNA Nano chip.

[1] To find out how much sample is lost in the first centrifugation, resuspend pellet in 500 ul of RNA lysis buffer, and repeat 3 previous steps. Label tube distinctly and proceed in parallel with the main sample.