

RNA PREPARATION FROM *ASPERGILLUS PARASITICUS*

1. Grind 100mg tissue in a chilled mortar and add to 1mL Trizol.
2. Incubate at least 5min at room temperature (RT).
3. Disrupt for 30sec in a Disruptor Genie.
4. Spin @ 12000g, 4°C for 10min.
5. Remove liquid to a new eppie; add 200uL Chloroform per mL Trizol.
6. Shake tube vigorously for 15sec.
7. Incubate tube @ RT x 2-3min.
8. Spin @ 12000g, 4°C for 15min (RNA in aqueous phase, should be colorless).
9. Transfer aqueous (~400uL) to a new eppie.
10. Add 250uL isopropanol, 250uL 3M NaOAc per mL of Trizol. Add 10ug (2uL) glycogen as a co-precipitate. Mix.
11. Incubate at RT x 10min.
12. Spin @ 12000g, 4°C for 10min.
13. Wash pellet with 1mL 75% EtOH. Vortex to dislodge pellet.
14. Spin @ 7500g, 4°C for 5min. Stand tube inverted for 20 min.
15. Dry pellet at 65°C for ~20min. (Remove EtOH with a pipet to dry faster)
16. Resuspend pellet in 400uL DEPC-dH₂O. Incubate at 65°C for 10min to aid resuspension.
17. Add 200uL 6M LiCl on ice for 2-4 hrs or 4°C overnight.
18. Spin at 14,000g for 5 min.
19. Pour off supernatant.
20. Wash pellet with 1 mL 75% EtOH and vortex.
21. Spin for 5 min at 7,500g.
22. Remove supernatant.
23. Air dry for 5-6 min.
24. Resuspend in 50-100uL DEPC H₂O + 1uL RNAsin.