Total RNA prep. by TRIzol

Invitrogen cat. # 155596-018

NOTE: Wear gloves at all times, use fresh tip boxes, new bag of microfuge tubes and all solutions should be RNase free.

- 1. Grind freeze dried mycelia with blue pipette tip.
- 2. In new microfuge tube, aliquot 50µL volume of ground mycelia powder
- 3. Add equal volume of acid-washed glass beads (particle size 425-600 µm) to each tube
- 4. Continue to grind with blue tip to a fine powder
- 5. Add 1mL of TRIzol reagent to each tube and vortex immediately for ~ 1 min.
- 6. Incubate at 15-30°C for 5 min.
- 7. Add 200µL of Chloroform (per 1mL of TRIzol used)
- 8. Shake vigorously for 15 sec.
- 9. Incubate at 15-30°C for 2-3 min.
- 10. Centrifuge at 12,000xg for 15 min at 2-8 °C
- 11. Transfer clear supernatant to new microfuge tube
- 12. Add 200µL of Chloroform (per 1mL of TRIzol used)
- 13. Shake vigorously for 15 sec.
- 14. Incubate at 15-30°C for 2-3 min.
- 15. Centrifuge at 12,000xg for 15 min at 2-8 °C
- 16. Transfer clear supernatant to new microfuge tube
- 17. Add 600µL Isopropanol(per 1mL of TRIzol used)
- 18. Mix by inversion and Incubate 15-30°C for 10 min
- 19. Centrifuge at 12,000xg for 10 min at 2-8 $^{\circ}$ C
- 20. Discard supernatant
- 21. Wash pellet with 1mL 70% EtOH (per 1mL of TRIzol used) **note: Ethanol should be made with DEPC H₂O**
- 22. Vortex
- 23. Centrifuge at 7,500xg for 5 min at 2-8 $^{\circ}$ C
- 24. Discard supernatant
- 25. Air dry under hood for 15-20 min. note: Do Not dry completely because solubility is decreased Do Not dry by centrifugation under vacuum
- 26. Resuspend pellet in 100μ L DEPC H₂O
- 27. Incubate in 65 °C water bath for 5 min to completely dissolve RNA

Yield: 5-10µg/µL RNA