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^{o}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^{o}C for 2-3 min.
10. Centrifuge at 12,000xg for 15 min at 2-8 ^{o}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^{o}C for 2-3 min.
15. Centrifuge at 12,000xg for 15 min at 2-8 ^{o}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^{o}C for 10 min
19. Centrifuge at 12,000xg for 10 min at 2-8 ^{o}C
20. Discard supernatant
21. Wash pellet with 1mL 70% EtOH (per 1mL of TRIzol used)
    note: Ethanol should be made with DEPC H_2O
22. Vortex
23. Centrifuge at 7,500xg for 5 min at 2-8 ^{o}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 100µL DEPC H_2O
27. Incubate in 65 ^{o}C water bath for 5 min to completely dissolve RNA

Yield: 5-10µg/µL RNA