

Total RNA Extraction-Trizol method

1. Add 1ml TRI reagent to fresh/frozen material in 1.5/2ml tube
2. Homogenise using small pestle/beads
3. Incubate at room temperature for 5 minutes
4. Add 0.2ml Chloroform and shake for 15 seconds
5. Leave for 5 minutes at room temperature
6. Centrifuge for 10 minutes at 12000 rpm at 4°C
7. Transfer upper aqueous phase to clean 1.5ml tube containing 0.5ml Isopropanol
8. Incubate at room temperature for 10 minutes
9. Centrifuge for 8 minutes at 12000rpm at 4°C, discard supernatant
10. Wash pellet with 1ml 75% EtOH
11. Centrifuge for 5 minutes at 7500rpm at 4°C
12. Resuspend in 30-50µl TE buffer or RNase-free water