

Trizol extraction of RNA from human muscle

1. Homogenize ~10-15 mg muscle in 500 ul Trizol at medium speed (15 sec on, 1 minute wait on ice, 3 times total). Can be indefinitely frozen at -80 C.
2. Allow samples to come to room temperature.
3. Add 100 ul chloroform . shake vigorously for 20 sec. Incubate 2-3 min at room temperature.
4. Centrifuge at 12,000 x g for 15 min at 4 C.
5. Transfer aqueous phase (~250 ul) to new tube. Be careful not to touch the interphase (white).
6. Mix RNA with 250 ul of isopropyl alcohol and incubate samples at RT for 10 minutes.
7. Centrifuge at 12,000 x g for 10 min at 4 C.
8. Remove supernatant completely. Wash RNA pellet once with 500 ul 75% EtOH. Vortex samples and centrifuge at 7500 x g 5 min at 4 C.
9. Repeat wash step.
10. Airdry RNA pellet for 5-10 minutes (be careful not to overdry).
11. Dissolve RNA pellet in DEPC water and measure concentration.

11 mg muscle yielded 1.6 ug RNA with A₂₆₀/A₂₈₀ ~1.95 and A₂₆₀/A₂₃₀ ~1.9