

TRIzol Method

This is a modification of the procedure originally described by Chomczynski P and Sacchi N. 1987. Signal-Step Method of RNA Isolation by Acid Guanidinium Thiocyanate –Phenol -Chloroform Extraction. *Analytical Biochemistry* 162: 156-159

©*Materials and Reagents*

TRIzol Reagent (Commercially available from many vendors)

Home-made recipe for 1 L:

Reagents	Final Concentration
Phenol in saturated buffer 380 ml	38 %
Guanidine thiocyanate 94.53 g	0.8 M
Ammonium thiocyanate 76.12 g	0.4 M
Sodium acetate, pH 5.0 33.4 ml of 3 M stock	0.1 M
Glycerol 50 ml	5 %
DEPC-Water Adjust the final volume to 1 L	

0.8 M sodium citrate / 1.2 M NaCl

Isopropanol (2-Propanol)

Chloroform

DEPC-Water

75% ethanol prepared with DEPC-Water

RNase Inhibitor (e.g. aseERASE™ – BIO 101 – Cat. 2601-104)

50 ml sterile plastic screw-cap centrifuge tubes

1. Grind 1g tissue in liquid nitrogen in a mortar and pestle.
2. Transfer powdered tissue to a 50 ml sterile plastic screw-cap centrifuge tube containing 15 ml TRIzol reagent. Incubate samples at room temperature or at 60°C for 5 min.
3. Homogenize tissue with homogenizer for 15 seconds. Repeat once.
4. Centrifuge samples at 12,000 x g at 4 °C for 10 min.
5. Transfer supernatant into new sterile 50 ml sterile plastic screw-cap centrifuge tube. Discard pellet.
6. Add 3 ml chloroform to each tube in hood. Shake tubes vigorously with vortex for 15 sec.
7. Let tubes sit at room temp 2-3 min. Centrifuge tubes at 10,000 x g at 4°C for 15 min.
8. Carefully pipet aqueous phase into a clean screw-cap centrifuge tube; discard interphase and lower phase into waste.
9. Precipitate RNA by adding Isopropanol and 0.8 M sodium citrate/1.2 M NaCl, half volume of the aqueous phase each. Cover tube and mix by gentle inversion. Let sit at room temperature for 10 min.
10. Centrifuge tubes at 10,000 x g at 4°C for 10 min. Discard supernatant.
11. Wash pellet with 20 ml of 75% ethanol. Vortex briefly.
12. Centrifuge at 10,000 x g at 4°C for 10 min. Discard supernatant; briefly dry pellet on kimwipe.

13. Add 100-250 μ l DEPC-Water, to pellet. Resuspend RNA by pipetting up and down a few times.
14. Add 1 μ l RNase inhibitor aseERASE to a 250 μ l RNA sample. If having problems resuspending the RNA pellet, we suggest incubation at 55 - 60°C for 10 min.
15. Transfer sample to microcentrifuge tube at room temperature.
16. Spin samples at high speed in microcentrifuge tube for 5 min at room temperature (to pellet the material that would not resuspend).
17. Transfer RNA solution (supernatant) to a new tube. Determine RNA concentration and quality by spectrophotometry.

Note: For optimal spectrophotometric measurements, RNA aliquots should be diluted with water or buffer with a basic pH. Water with pH < 7.5 falsely decreases the 260/280 ratio.