

ISOLATION OF TOTAL RNA FROM 10 CM² PLATES

Materials: TRIzol Reagent InVitrogen cat#15596-026 (100mL) Scrapers (sterile, RNase free), Syringes and 20g needles, sterile 15ml polypropylene tubes or sterile microfuge tubes.

Cell Culture:

- (3) 10cm plates for Preadipocyte time points
- (2) 10cm plates for early differentiation time points
- (1) 10cm plates for Adipocytes

The Cell Harvest:

1. Aspirate media from plates.
2. Wash plates once with ice cold 1x PBS and aspirate off PBS
3. Add 1ml TRIzol reagent per plate.
4. Scrape cells with cell scraper and pool cell mass into corner of plate
5. Triturate cell suspension with 20g needle on a 5ml sterile syringe.
6. The lysate is then transferred to the second plate and the procedure repeated. And so forth with the third plate.

Example: 3 ml of TRIzol on the first plate, scraping the cell mass to one corner, then pipetting it up and down using a sterile syringe with a 20-gauge needle. This insures lysis of the cells and shears the DNA. Then transfer to next plate.

7. Resulting suspension is 3 plates in 3 ml, which is then transferred to a 15 ml polypropylene tube and stored at -80° until the time of RNA isolation.

RNA Isolation

1. Thaw homogenates and aliquot out 1ml into 1.5ml microfuge tubes
2. Incubate at room temp for 5minutes
3. Add 200ul Chloroform, vortex into single phase & incubate for 3min @RT
4. Centrifuge 12k x g for 15min @ 4°C
5. Transfer aqueous phase (top layer) to clean tube
6. Optional: second chloroform extraction to remove residual phenol
7. Add 500ul Isopropanol and vortex
8. Incubate 10 min at room temp (can be froze -80°C o/n) precip. often visible
9. Centrifuge 12k x g for 10min @ 4°C

10. Remove supernatant
11. Wash with 1ml 75% ethanol (add to tube and gently vortex)
12. Centrifuge 7.5k x g 5min @ 4°C
13. Aspirate off supernatant
14. Air dry pellet (pellet is white while wet and dries clear, don't over dry)
15. Resuspended pellet in 70ul of nuclease free dH₂O
16. Incubate 10 min @ 55°C
17. Place on ice
18. NanoDrop for concentration
19. Store -80°C