

Trizol RNA Isolation

Collect cell pellets according to standard protocol.

1. Add 1mL trizol (per 10cm plate) to cells, and lyse by repetitive pipetting & vortexing. Incubate homogenized samples for 5min at RT. (use more trizol for larger cell pellets)
2. Add 200 μ L chloroform, vortex 15sec, and incubate again at RT 2-3min. Centrifuge samples @ 12,000 $\times g$ for 15min @ 4°C. (for larger cells pellets use 200 μ L chloroform for every mL trizol)
3. Collect the colorless, upper liquid phase and add to 0.5mL trizol, vortex and incubate 5min @ RT.
4. Add 100 μ L chloroform, vortex 15sec, and incubate again at RT 2-3min. Centrifuge samples @ 12,000 $\times g$ for 15min @ 4°C.
5. Collect the colorless, upper liquid phase and precipitate with 0.75mL isopropanol. (0.5mL isopropanol per mL trizol) Incubate samples at RT for 10min, and centrifuge at 12,000 $\times g$ for 10min at 4°C.
6. Wash RNA pellet 1-3X w/ 75% ethanol, (1mL EtOH per 1mL trizol). Mix by vortexing and centrifuge @ 7,500 $\times g$ for 10min at 4°C.
7. Remove all EtOH, and air-dry ~5min. Resuspend RNA in appropriate buffer.