**Trizol RNA extraction**

1. Wash cells in 35mm dish 2x with PBS (wash= add ~1ml PBS and aspirate).
2. Lyse cells directly in a culture dish by adding 1 mL of TRIZOL Reagent to the dish, and passing the cell lysate several times through a pipette. Use a rubber policeman to scrape the bottom of the dish so all the cells come off. Transfer the TRIZOL/lysed cells to a 1.5ml centrifuge tube. After transferring the majority of the liquid, hold the dish at a 45 degree angle use a rubber policeman to scrape all the liquid to the bottom of the dish and transfer this residual TRIZOL/lysed cells as well.
3. Incubate the hom­ogenized samples for 5 minutes at room temperature to permit the complete dissociation of nucleoprotein complexes. **NOTE:** at this stage, the suspension can be stored at -80°C before continuing.
4. Add 0.2 mL of chloroform per 1 mL of TRIZOL Reagent **(NOT chloroform:isoamyl alcohol mix**). Cap sample tubes securely.
5. Shake tubes vigorously by hand for 15 seconds, incubate @ room temperature for 2 to 3 minutes.
6. Centrifuge @ 12,000xg, 15 minutes, 4°C. *Following centrifugation, the mixture separates into a lower red, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase.*
7. Transfer the aqueous phase to a fresh tube
8. precipitate the RNA from the aqueous phase by mixing with isopropyl alcohol. Use 0.5 mL of isopropyl alcohol per 1 mL of TRIZOL Reagent used for the initial homogenization.
9. Incubate samples at room temperature for 10 minutes and centrifuge at no more than 12,000 x g for 10 minutes at 4°C. The RNA precipitate, often invisible before centrifugation, forms a gel-like pellet on the side and bottom of the tube.
10. Carefully remove as much of the supernatant as possible. Wash the RNA pellet once with 75% ethanol, adding at least 1 mL of 75% ethanol per 1 mL of TRIZOL Reagent used for the initial homogenization.
11. Mix the sample by vortexing and centrifuge at 7,500 x g for 5 minutes at 4°C.
12. Carefully remove as much of the supernatant as possible and dry the pellet in the speedvac for 5-10 min. Try not to overdry (i.e. don’t forget your sample and leave it in there for a half hour).
13. Dissolve RNA by adding 50ul nuclease-free H2O and incubate the tube at 60°C for 10 min. **NOTE: The RNA is EXTREMELY sensitive to RNase at this phase. Be careful to not touch the rim of the microfuge tube or allow any kind of water droplets near the tube openings as droplets contain lots of RNase and your sample will be degraded quickly.**
14. After dissolving, make sure to keep tube on ice and store RNA at -80°C.
15. Use Nanodrop to calculate [RNA].