

<b>Title</b>	<b>RNA Extraction From mammalian cells</b>		
<b>Protocol Number</b>	MOLBIO20001	<b>Written by</b>	Duha A.
<b>Adapted from</b>	MO Lab and <a href="#">thermo fisher</a>	<b>Version</b>	1.0
<b>Date created</b>	15 June 2020	<b>Version update</b>	NA

Make sure the centrifuge is at 4°C before you start the extraction

**Reagents:**

- TRIzol
- Chloroform
- Isopropanol
- 75% ETOH
- RNAase free water

**Protocol:**

1. To the eppendorfs containing TRIzol lysed cells, add 200µL of chloroform per 1mL of TRIzol.
2. Mix by inverting the tube several times
3. Incubate for 2-3 mints at RT
4. Centrifuge the content of each eppendorf at 10,800 RPM at 4°C for 15 mint.
5. Transfer the RNA (which is in the supernatant of the tube) without touching the white pallet into a new Eppendorf
6. If the sample is small, add 1 µL (or between 5-10µg) of RNase-free glycoblu.
7. Add 500µL of isopropanol for 1mL of TRIzol used during cell lysis
8. Incubate for 10 mints at RT.
9. You can store the the sample at -80°C or continue.

**To continue:**

10. Centrifuge at 10,800 RPM at 4°C for 15 mint. RNA will precipitate into a white pallet
11. Remove the supernatant using a pipette
12. Wash the pallet with 1mL of 75% ETOH for every 1mL of TRIzol
13. The RNA can be stored in 75% ethanol for at least 1 year at -20°C, or at least 1 week at 4°C

**To continue:**

14. Vortex the sample briefly then centrifuge for 5 mints at 8100 RPM at 4°C
15. Remove supernatant using a pipette.
16. Allow the pallet to airdry for 5-15 mints

**IMPORTANT!** Do not dry the pellet by vacuum centrifuge. Do not let the RNA pellet dry, to ensure total solubilization of the RNA. Partially dissolved RNA samples have an A230/280 ratio <1.6.

17. Resuspend the pallet in 20-50µL of RNAase free water by gently pipetting up & down.
18. Incubate in a water bath or heat block set at 55-60°C for 10-15 minutes.
19. Dose the RNA using the nanodrop. Blank with RNAase free water and use 2µL of sample for measurement.
20. Record the 260/280 and 230/260 ratios and the RNA quantity in µg.
21. Proceed to downstream applications, or store the RNA at -80°C