

HOT PHENOL RNA EXTRACTION PROTOCOL

- 1) Set the water bath to 80°C.
- 2) Make 50 mL Extraction buffer as follows (in a baked bottle):

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| 1M Tris-HCl (pH = 8.0) | = 5 mL |
| 8M LiCl | = 0.625 mL |
| 0.5M EDTA (pH = 8.0) | = 1.0 mL |
| SDS | = 0.5 gm |
| DEPC treated water | = 43.2 mL |
| Total | = 50 mL |
- 3) Add 50 mL phenol (pH = 4.7), in 50 mL extraction buffer (final concentration of 1:1). For small sample add 200 to 300ul of 1Extraction buffer: 1Phenol.
- 4) Heat the above mixture to 80°C for at least 1/2 half.
- 5) Grind the tissue in 1.5 mL Liquid Nitrogen and immediately add 15mL (for 1.5gm of tissue), for extraction in eppendorf tubes add 750 µL of 1Extraction buffer: 1Phenol. If the tissue is hard use mortar and pestle.
- 6) Homogenize by vigorous vortexing.
- 7) Add 350µL of chloroform to the samples and vigorously vortex again.
- 8) Centrifuge for 15 min @14000 rpm at RT.
- 9) Transfer the supernatant to 1.5 mL new Eppendorf tube.
- 10) Add 1/3 volume of 8 M LiCl to the supernatant and mix well by inverting the tubes.
- 11) Precipitate on ice for at least 2 hrs.
- 12) Balance the tubes if needed using 2 M LiCl and centrifuge at 10,000 rpm for 30 mins at 4°C.
- 13) Discard the supernatant and wash the RNA pellet with 500 µL of 2M LiCl (RT) and centrifuge at 10,000 RPM for 5 min (RT).
- 14) Discard the supernatant and wash the RNA pellet with 500 µL of 70% DEPC treated EtOH. Centrifuge again at 10,000 RPM for 5 min (RT).
- 15) Discard the EtOH and air dry the pellet.

- 16) Dissolve the RNA in 100 μ L of DEPC treated water. If it does not dissolve immediately, put the tubes in -80°C and then thaw on ice after 2 hrs. You can also put the tubes in -80°C overnight.
- 17) Centrifuge at 10,000 RPM for 10 min at 4°C and transfer the supernatant to a new tube. This step is to get rid of any un-dissolved debris.
- 18) Add 1/10th vol. of sodium acetate (3M, pH-5.2) and 2.5vol. of 100% EtOH (RT). (sodium acetate = 0.1 and EtOH = 2.5 vol).
- 19) Precipitate the RNA at -80°C for 20 min and centrifuge at 10,000 RPM for 15 min at 4°C .
- 20) Discard the supernatant and wash the pellet (RT) with 70% EtOH and centrifuge again at 10,000 rpm for 5 mins at 4°C .
- 21) Air dry the pellet properly.
- 22) Dissolve the RNA in 50 μ L of DEPC treated water and store it in -80°C .
- 23) Treat the samples with DNase 2 μ L and incubate at 37°C water bath for 1/2hr.
- 24) Stop the enzyme reaction by adding 10 μ L of 20mM EGTA stop solution and incubate at 65°C for 10 min.
- 25) Quickly run normal 1.5% agarose gel to see the quality and quantify the RNA.
- 26) Extract RNA by adding equal volume of 1phenol:1chloroform, mix well and then centrifuge at 14000 rpm at RT for 10min.
- 27) Add 1/10 vol of Sod acetate (pH 5.2) and 2.5 vol of 100% ethanol, mix well and centrifuge at 14000 rpm for 10 min.
- 28) Wash the pellets with 70% ethanol (DEPC water) and centrifuge at 14000rpm for 5min,
- 29) Drain the ethanol and air dry the pellets, depending upon the size of pellet /quantification dissolve in DEPC treated water and store at -80°C .
- 30) Proceed for cDNA synthesis.