

Trizol Extraction (GibcoBRL)

NOTE: CHANGE GLOVES OFTEN THROUGHOUT PROCEDURE!!!!

Sample Needed:

- RNA in aqueous layer of isopropyl alcohol from first portion of TRIzol extraction.

Materials Needed:

- Buffer RLT (recommended to make sure b-ME is added to Buffer RLT before use, however this practice is not employed in our lab), stored in drawer of RNase free bench
- DEPC (RNase free water), stored in drawer of RNase free bench
- 75% RNA-only Ethanol (RNase free bench or chemical cabinet)
- RNA-only Isopropyl alcohol (chemical cabinet)
- 1.5ml collection tubes (RNase free)
- Quiagen RNeasy Mini-Columns

Equipment:

Use either eppendorf 5415C-centrifuge in glass door fridge(for spins at 4-8°C) or on RNase free bench (for spins at RT). The procedure should be performed in a fume hood, except where noted. Label all tubes prior to administering sample to avoid confusion.

1. Incubate samples at -20°C overnight.
2. Centrifuge at 12,000g for 15 minutes at 2-8°C (setting 14 on eppendorf 5415C in glass door fridge). Set tubes with back to outside of centrifuge for easy pellet location. Remove from centrifuge ASAP and proceed directly to next step.
3. Remove the supernatant by pouring eluate away from pellet into fresh 15-ml conical (just in case RNA pellet dislodges). Wash the RNA pellet once with 75% ethanol, adding at least 1ml of 75%EtOH for every 1ml of TRIzol (in initial homogenization). Place tips in general hazardous waste bucket.
4. Mix sample by vortexing. You may see a bit of a pellet remaining; that's okay.
5. Centrifuge at 7500g (9458 rpm) for 5 minutes at 2-8°C. During this spin prepare drying rack (Accuwipe stuck in row of tube rack) and label mini-columns.
6. After spin, dump supernatant into Trizol/EtOH hazardous liquid waste bucket. Briefly (1-2 minutes) dry the RNA pellet on drying rack. (DO NOT ALLOW TO COMPLETELY DRY OR RNA PELLETS WILL NOT REDISSOLVE.) Also can dry by just placing upside down on blue absorbent pad.
7. Add 100 λ of DEPC (RNase free water). Pipet to get pellet into solution (probably at least 30 times). Watch pellet carefully as to not lose it on the side of the pipet tip.
8. Add 350 λ Buffer RLT and pipet to mix thoroughly. Use RLT waste bucket for pipet tips.
9. Add 250 λ ethanol (96-100%) to the diluted RNA and mix thoroughly by pipetting. Do not centrifuge.
10. Apply the sample (about 750 λ) to an Rneasy mini-column placed in a 2ml collection tube. Close the tube gently and centrifuge for 15 seconds at 10,000rpm at RT (just hold down button on machine and release to stop). Discard flow through in Buffer RLT liquid waste and collection tube/pipet tips in Buffer RLT solid waste.
11. Transfer the Rneasy column into a new 2ml collection tube. Pipet 500 λ Buffer RPE onto the Rneasy column.

12. Close the tube gently and centrifuge for 15seconds at 10,000rpm (dial at 10) at RT to wash the column. Discard the flow-through in Buffer RLT liquid waste. No need to switch micro-centrifuge tubes.
13. Add another 500 λ Buffer RPE to the RNeasy column. Close the tube gently and centrifuge for 2 minutes at 10,000rpm to dry the RNeasy silica-gel membrane.
14. Place the RNeasy column in a new 2 ml collection tube and discard the old collection tube with the flow-through in solid and liquid RLT hazardous waste, respectively. Centrifuge in a microcentrifuge at full speed for 1 minute.
15. To elute, transfer the Rneasy column to a new 1.5 ml collection tube. Pipet 30 λ of DEPC directly onto the Rneasy silica-gel membrane. Close the tube gently and centrifuge for 1 minute at 10,000rpm at RT to elute.
16. Work on RNase free bench from now on. Pour eluate over the column again to ensure collection. Centrifuge for 1 minute at 10,000rpm to elute. Dispose of the column into non-hazardous bench top waste container. Label the tubes with patient ID, date, prep, and initials. Place in RNA storage box in -80°C until ready for cDNA prep.