

Total RNA Isolation/Purification from *Toxoplasma gondii* Infected NIH3T3 Cells

Notes:

- **Solutions must be RNase Free!!**
- To achieve this make sure that all solutions (except those containing Tris) have been treated with DEPC (diethyl polycarbonate), or purchase and certified RNase and DNase free.
- Change gloves regularly, and use filtered pipette tips.
- This method creates RNA free from proteins and carbohydrates. This is Suitable for microarray use, whereas other RNA isolation techniques might not be.
- Some parts need adjusting for numbers of cells, particularly column/kit used
- I usually have 1 x T75 flask of infected cells per midi column in about 5ml of TRI reagent. I also usually take this up in 50-100 μ l of water in step 24.
- RNeasy mini kits may be used and volumes should be adjusted for smaller flask sizes and other cells types.

Experiment:

1. To a flask of infected or control cells add 5 ml TRI Reagent (Sigma) solution cells.
2. Mix and pipette into clean 50 ml Falcon tube
3. The samples can be used immediately or frozen and stored at -80°C for up to a month.
4. Remove tube containing cells from the freezer, and let stand at room temperature (RT) for 5 minutes.
5. In a fume hood, add 1 ml chloroform to a 14ml snap cap falcon tube (0.2 ml of Chloroform per 1 ml of TRI Reagent).
6. Shake vigorously for 1 minute and incubate at RT for 3 minutes.
7. Place the tubes into rubber adaptors for Sorvall SS34 rotor (or equivalent). Centrifuge samples at 4°C at 10,000 rpm for 15 mins.
8. Carefully transfer the aqueous phase to a fresh snap cap tube. **Do not touch/disturb the interface or organic phase!!**
9. While gently vortexing the new tube containing the aqueous phase, add drop-wise 2.5 ml propan-2-ol (0.5 ml per 1ml TRI Reagent) incubate for 15 minutes at RT.
10. In this time organize the appropriate numbers of Qiagen RNeasy Midi columns.
11. Transfer the solution to a Qiagen affinity column and centrifuge for 5 minutes at 4000 rpm.
12. Vortex the flow through and add to the affinity column again. Centrifuge for 5 minutes at 4000 rpm.
13. Repeat Steps 11 and 12 if necessary.

14. Discard the flow through, and add 2.5 ml of Buffer RPE (supplied) to the column. Centrifuge for 2 minutes at 4000 rpm.
15. Discard the flow through and add another 2.5 ml of Buffer RPE to the column. Centrifuge for 5 minutes at 4000 rpm.
16. Place the column in a new 15 ml collection tube (supplied).
17. To elute the RNA, add 150 μ l of RNase-free water (supplied) to the column and centrifuge for 3 minutes at 4000 rpm.
18. Repeat step 16 twice more, and as the samples are the same use the same collection tube.
19. Transfer flow through to a 2 ml Eppendorf tube.
20. Add 45 μ l 3M sodium acetate (pH 5.2-5.3) and 1125 μ l of 100% ethanol, vortex and place on ice for 10 minutes.
21. Centrifuge the tube for 15 minutes at 12,000 rpm at 4°C.
22. Pour off supernatant and add 200 μ l 75% ethanol in RNase free water. vortex vigorously, and centrifuge for 10 minutes at 12,000 rpm at 4°C.
23. Pour off 75% ethanol and place in the speed vac to dry at 65°C for 10 minutes or until dry.
24. Add 10-20 μ l of RNase-free water drop-wise avoiding pellet and place at 65°C for 10 minutes (or until dissolved). **Do not mix as pellet may stick to tip!**
25. Centrifuge out debris at 13,000 rpm for 5 minutes. Place supernatant into new tube and quantitate using a spectrophotometer.
26. Aliquot into samples of according to need (usually 30-40 μ g total for microarray analysis) and store at -80°C until use.