

Experiment: _____

Date: _____

RNA Isolation from Tissue Culture Cells w/ Trizol

Plate ID	Day	% Diff'd	Appearance of Plates & Other Notes

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*** KEEP ON ICE AT ALL TIMES TO AVOID RNA DEGRADATION***

You Will Need:

- Chilled Trizol Reagent (Invitrogen)
- Sterile 5 or 10 ml pipettes
- 14 ml pop-cap Falcon Tubes ← All Reagents, tips, etc should be designated RNA ONLY!
- Ice Bucket

- 1) All steps done in the hood since working with Trizol!
- 2) Place plate of ice. Aspirate off all media. DON'T RINSE!
- 3) Add 3 ml Trizol per plate. Pipette Trizol around the plate multiple times to get all the cells. (Can see when cells have come off bottom.)
- 4) Transfer to 14 ml Falcon tube. Pool two plates per tube to ensure adequate RNA concentration.
- 5) Vortex well and place immediately on ice.
- 6) Repeat for each plate. When all plates done, begin isolation or store for short period (o/n but not much more) @ -20C or, if must be for longer, -80 C.

Isolation:

You will need:

- Chloroform (pre-chilled)
- Isopropanol (pre-chilled) ← All Reagents, tips, etc should be designated RNA ONLY!
- 70 % EtOH (pre-chilled)
- DEPC-H₂O

- 7) Vortex cells @ max for 2 minutes.
- 8) Incubate on ice for 5 minutes.
- 9) Add 1 ml pre-chilled chloroform. Vortex 1 minute. Incubate on ice 5 mins.
- 10) Spin @ 10K for 10 mins on 4C Sorvall with SS34 rotor.
- 11) Trx upper phase carefully to new falcon tube. (It is better to leave some of the clear upper phase behind in the tube than to risk picking up interphase!)

- 12) One tube at a time, add equal volume chilled isopropanol. Invert tube 5x. Vortex lightly. Incubate tubes on ice for 10 minutes.
- 13) Spin @ 10K for 10 mins.
- 14) Discard supernatant. Wash pellet with 5 ml 70% EtOH. Pipette up and down well. Spin 8K 10 minutes.
- 15) Remove supernatant. Wash again with 1 ml 70% EtOH. Transfer to eppendorf and spin at max rpm in 4C microfuge, 5 minutes. Leave as an EtOH ppt at -80C until ready to use. Then...
- 16) Spin down again. Remove supernatant and air dry pellet. Resuspend in ___ ul DEPC- H₂O. Store @ -80C. (Use appropriate volume for your needs: cDNA rxn, RT-PCR, etc etc).