

Procedure 1.1.1 - RNA Isolation from Cell Culture

Date: \_\_\_\_\_

Experiment ID: 1.1.1-*yyyyyuuuuuu*

Technician: \_\_\_\_\_

Project: \_\_\_\_\_

Client Signature: \_\_\_\_\_

1. Aspirate media from plate(s). \_\_\_\_\_
2. Wash cells with 10mls PBS (w/o Calcium and Magnesium). \_\_\_\_\_
3. Dispense 1mL Trizol per cm of plate diameter onto plate(s). \_\_\_\_\_
4. Lean the plate forward and repeatedly pipette the Trizol onto the cells until they have all lysed and the Trizol is homogenous.
5. Transfer the lysate to an appropriately labeled conical tube.
6. Pellet a Phase-Lock tube by centrifuging at 4000rpm for 5 minutes. \_\_\_\_\_
7. Add 0.2 Trizol-volumes of chloroform to the tube and vortex. \_\_\_\_\_
8. Immediately transfer to the phase-lock tube.
9. Incubate at room temperature for 15 minutes.
10. Centrifuge at 4000rpm for 15 minutes at room temperature. This will separate the phases and the phase-lock will form a barrier between the aqueous upper phase and the organic lower phase.
11. Decant the upper phase into a new conical tube containing 0.5 Trizol-volumes of Isopropanol. \_\_\_\_\_
12. Vortex vigorously and incubate at room temperature for 15 minutes.
13. Centrifuge at 4000rpm for 1 hour at 4C to pellet the RNA.
14. Wash the pellet with 1 Trizol-volume ice-cold 75% ethanol by centrifuging for 15 minutes at 4000rpm. \_\_\_\_\_
15. Decant the ethanol and centrifuge again for 5 minutes.

16. Remove the residual ethanol with a pipettor. \_\_\_\_\_
17. Immediately resuspend the pellet in 800ul nuclease-free water. \_\_\_\_\_
18. Add 400ul 1.5M LiCl solution. \_\_\_\_\_
19. Vortex vigorously and incubate at -20C for at least 2 hours.
20. Centrifuge at maximum rcf for 15 minutes.
21. Wash pellet with ice-cold 75% ethanol twice. \_\_\_\_\_
22. Remove residual ethanol with a pipettor and immediately resuspend in 250ul nuclease-free water. \_\_\_\_\_
23. Determine RNA concentration using the NanoDrop spectrophotometer (Appendix 7.1). It will generally be necessary to concentrate the RNA at this point. Determine the target volume that will place the RNA at the desired concentration. \_\_\_\_\_
24. Add 28ul 3.0M NaOAc. \_\_\_\_\_
25. Add 700ul 100% EtOH and vortex vigorously. \_\_\_\_\_
26. Incubate at -20C for at least 15 minutes.
27. Centrifuge at maximum rcf for 15 minutes at 4C.
28. Wash the pellet with 75% EtOH as described in steps 21 and 22 but resuspend in half the volume calculated in step 23. \_\_\_\_\_
29. Measure RNA concentration and add nuclease-free water to dilute the RNA to the desired concentration. \_\_\_\_\_
30. Verify RNA integrity (BioAnalyzer, Appendix 7.2). \_\_\_\_\_
31. Store RNA as described in procedure 1.2