**RNA Extraction Protocl (Trizol Plus Pure-link Column)**

RNA Extraction:

1. Clean area with 70% ethanol or NaOH solution and then with RNAse Away
2. Clean pipettes similarly
3. Set centrifuge settings at 4°C (12,000 x g for 15 minutes)
4. Prepare Wash Buffer II in EtOH by adding 60mL to the Tube
5. Take Frozen Tissue and quickly pipette 900 mL of Trizol into its tube
6. Sonicate at ~25-30Hz until tissue is thoroughly homogenized
7. Incubate at RT for 5 minutes
8. Add 200uL of Chloroform and shake tube vigorously for 15 seconds (Do not Vortex)
9. Incubate for 3min
10. Centrifuge at 12,000xRCU at 4°C for 15 minutes
11. Pipette upper colorless phase into a sterile Eppendorf Tube (~500uL)
12. Add equal volume of 70% EtOH
13. Vortex and after invert the tube
14. Transfer solution into a Spin Cartridge with a Collection Tube
15. Centrifuge at 12,000xRCU at RT for 15 seconds
16. Discard the flow-through and re-insert the Spin Cartridge into same Collection Tube
17. Repeat until all of the sample has been processed
18. Add 700uL of Wash Buffer I to spin-cartridge
19. Centrifuge at 12,000xRCU at RT for 15 seconds
20. Discard flow-through and insert spin-cartridge into new collection tube
21. Add 500uL of Wash Buffer II to spin-cartridge
22. Centrifuge at 12,000xRCU at RT for 15 seconds
23. Discard flow-through and insert spin-cartridge into same collection tube
24. Repeat ONCE
25. Centrifuge the spin-cartridge and new collection tube for 60 seconds at 12,000xRCU
26. Discard the collection tube and insert spin-cartridge into recovery tube
27. Elute with 30uL of Elution buffer (RNAase free Water) to center of spin-cartridge
28. Incubate at RT for 60 seconds
29. Centrifuge at 13,000xRCU for 2 min at RT
30. Discard spin-cartridge, and transfer the pure RNA in the recovery tube to a low-retention tube for long-term use.
31. Check Purity and concentration in NanoDrop (~2.0 A260/A280)
32. Normalize all of the pure RNA prior to reverse transcription reaction.