

## RNA Extraction NDJ V.3

**\*\*Use RNAase on gloves and bench spaced\*\***

1. Get ice/Trizol/ RNAse free pippet
2. Put cerebella on ice and get a waste container for Trizol. (For cell culture, see below\*).
3. Add 0.5ml Trizol (use filter tips)
4. Homogenize cerebella
  - a. Initially smash by hand
  - b. Follow with Motor Pestle until completely liquidify
5. Add 0.5ml Trizol again
  
6. Centrifuge 10 min 4°C 12,000xg
7. Get RNA-free tube. Close and label
8. Pippet supernatant into new tubes
9. Sit 5 min RT (room temperature)
10. Add 200ul Chloroform (Not CIAA) Out of molecular biology grade bottle
11. Shake by hand 15seconds
12. Incubate 2-3minute RT
13. Centrifuge 15 minute 4°C 12000xg
14. Put on ice and put aqueous top layer into clean RNAse-free tube.
15. Add 500ul Isopropanol (2-propanol; RNA only bottle).
16. Incubate 10 minute RT
17. Spin 10 minutes 4°C 12000xg
18. The RNA should form a visible pellet
19. Remove supernatant. Use a small pipette to remove the liquid around the pellet
20. Add 75% EtOH (RNAse free). Pipette pellet off tube.
21. Centrifuge 5 min 4°C 7500xg
22. Remove all of supernatant. Use small pipette again to remove small droplets
23. Turn upside down and let air dry.
24. Add 20-40ul nuclease free H<sub>2</sub>O.
25. Store -80°C

\*For cell culture extraction, add 1ml trizol to 3.5cm dish(1ml/10cm<sup>2</sup>). Pippette trizol on cells and swirl. Pippetted up and down 3 times—moving the cellular material to oneside. Continue with step 6.