RNA Extraction from Tissue

References

1. Lang, A. E., Riherd Methner, D. N., & Ferreira, A. (2014). Neuronal degeneration, synaptic defects, and behavioral abnormalities in tau_{45-230} transgenic mice. Neuroscience, 275, 322–339. https://doi.org/10.1016/j.neuroscience.2014.06.017
2. Kelly, B. L., Vassar, R., & Ferreira, A. (2005). Beta-amyloid-induced dynamin 1 depletion in hippocampal neurons. A potential mechanism for early cognitive decline in Alzheimer disease. The Journal of biological chemistry, 280(36), 31746–31753. https://doi.org/10.1074/jbc.M503259200

Protocol

1. Day Before: Supply Preparations.
    a. Obtain (1) 100 ml bottle for ethanol, (1) 100 ml graduated cylinder, (# of samples) mortar/pestle homogenizer sets, (1) small weighing spatula.
    b. Wash all thoroughly with dH_20.
    c. Glass Objects: Incubate in oven overnight.
    d. Plastic Objects: Incubate 10 minutes in 0.5 M Sodium Hydroxide, rinse thoroughly and wrap in aluminum foil.
2. Day of: autoclave all instruments and materials.
3. Make 75% Ethanol: In sterilized 100 ml graduated cylinder, make 75% RNAse free Ethanol with DEPC-water and store in sterilized bottle.
4. Sample Homogenization (Keep samples on ice):
    a. Using sterile spatula, remove tissue (frozen) from Eppendorf tube and place in sterile glass homogenizer
    b. Add 1 ml Trizol to tissue
    c. Homogenize with ~10 strokes
    d. Transfer into RNAse free tube
    e. Incubate 5 minutes at room temperature
5. Add 200 µL Chloroform, cap, and shake in holder vigorously for 15 seconds.
6. Incubate at room temperature for 3 minutes.
7. Centrifuge samples at 12,000 x g for 15 minutes at 4°C.
8. Collect aqueous supernatant and place into new RNAse-free tube.
9. Precipitation: Add 500 µL Isopropanol to aqueous phase.
10. Mix by gently inverting holder with samples 4-5 times.
11. Incubate at room temperature for 10 minutes.
12. Centrifuge at 12,000 x g for 15 minutes at 4°C.
13. Carefully remove supernatant and dispose.
14. Wash pellet in 1 ml 75% Ethanol (in DEPC-H_20).
15. Mix by vortexing and spin at 7,500 x g for 5 minutes at 4°C.
16. Carefully remove and dispose of supernatant and air dry pellet for 5 minutes.
17. Dissolve pellet in 50 µL of DEPC-H_20 and mix gently.
18. Incubate at 55°C for 10 minutes.
19. Obtain concentrations and store at -20°C.