RNA extraction from tissues with TRIzol

Protocol JJ1
Last modified: 180517
Based on: TRIzol Reagent User Guide (Cat. Numbers 15596026 & 15596018)

Notes:
- TRIzol is usually kept in the nucleic acids extraction room of KATZ
- Only human samples should be processed inside the extraction room
- If samples are extracted outside the extraction room, always work under a fume hood when handling TRIzol
- Dispose phenolic residues properly
- This protocol is also suitable for extracting RNA from fluids, but TRIzol LS should be used then

1. If samples were preserved in RNAlater, centrifuge samples at 3,000 x g for one min and pipette out RNAlater. If samples are fresh, snap-freeze tissues by submerging tubes in liquid nitrogen and ground samples to a powder using a Kontes pestle.

2. Add 1 ml of TRIzol to samples and macerate them with a Kontes pestle (if not previously ground to powder in liquid nitrogen). Max. amount of tissue here should be 100 mg. Make sure samples are properly homogenized. **Incubate 5 min at RT to allow for proper dissociation of ribonucleoprotein complexes.**

3. Add 0.2 ml of chloroform per ml of TRIzol. Make sure tubes are properly capped and **briefly vortex to homogenize.** Incubate for 3-5 min at RT.

4. Centrifuge 15 min, 12,000 x g, 4C.

5. Carefully transfer the supernatant to a new 1.7 ml tube trying to avoid touching the interphase (usually 500 ul are recovered).

6. Add 1 volume of isopropanol and incubate 10 min at RT (can also be longer at -20C if needed).

7. Centrifuge as in (4) but only 10 min.

8. Add 1 ml of 75% EtOH (RNA can be stored here for long periods at -20C). Vortex and centrifuge at 12,000 x g for 5 min at 4C. Discard supernatant with a pipette. Air dry the pellet for 10 min at RT.

9. Resuspend the pellet in 20-50 ul of RNase-free water. Heat samples at 60C for 5 min. Add 1 ul of RNaseout.