

**Trizol RNA isolation protocol (Modified by D. Francis <francis.77@osu.edu> from Edgar Huitema <huitema.1@osu.edu>)**

**Preparation:**

Make sure all your equipment and solutions are RNase free. DEPC treated solutions are highly recommended. Ambion also makes some products that inhibit RNase activity (RNA secure and RNase inhibitor).

Always use gloves to protect the RNA from you and you from the Trizol. Note MSDS for Trizol. This is a Phenol based product. A lab coat and safety glasses are recommended.

After recovery of the aqueous phase, keep samples on ice as much as possible.

**Protocol:**

- 1 Grind <100 mg of plant tissue in liquid nitrogen to a fine powder and transfer the material to the Trizol extraction buffer. You can use up to 100 mg of tissue per ml of Trizol.
- 2 Homogenize the suspension by shaking moderately for several seconds. Incubate 2-3 minutes at room temp. (20-30 C).
- 3 Add 0.2 ml chloroform for each 1 ml of initial Trizol. Shake for 15 seconds, and incubate for additional 2-3 min. at room temp.
- 4 Centrifuge the samples for 20 min. at 2000 RPM using a microfuge.
- 5 Remove the aqueous phase and transfer 550 ul to an eppendorf tube for each 1 ml of Trizol. Approximately 600  $\mu$ L can be recovered but for DNA contamination prevention, 550  $\mu$ L is recommendable.
- 6 Add 1 volume of phenol:chloroform:isoamyl alcohol (25:24:1) to the aqueous phase, mix well by vortexing and spin for appr. 10 minutes at maximum speed using a microfuge.
- 7 Take off aqueous phase and put that into a tube containing an equal volume of chloroform, spin at same conditions and transfer aqueous phase to a new tube.
- 8 Add 0.25 ml isopropanol and 0.25 ml of 1.2 M Na-citrate/ 0.8 M NaCl to each eppendorf tube. Invert the tubes to mix and let sit 2-3 min.
- 9 Spin the tubes in a microfuge at maximum speed for 10 min.
- 10 Take off the supernatant and wash the pellet with 1 ml filter sterilized 70% ethanol.
- 11 Dry the pellet in air (preferably in a sterile hood). Avoid completely drying the pellets as this will decrease the solubility of the RNA.
- 12 Dissolve the RNA in 50  $\mu$ l of sterile water or TE 1/10E and keep it in the freezer (-20 or -80 C) until further use. After recovery of the aqueous phase, keep samples on ice as much as

