



Trizol RNA isolation protocol for miRNA assays

Preparation:

Use TrIzol from Invitrogen (Catalog # 15596-026). Make sure all your equipment and solutions are RNase free. RNase free solutions are highly recommended. Always use gloves to protect the RNA from you and you from the TRIzol. This is a Phenol based product. After recovery of the aqueous phase, keep samples on ice as soon as possible.

Protocol:

1. Tissue (50-100mg) is dissected and “chopped” roughly using a razor blade. Add 1ml TRIzol solution and transfer into an eppendorf tube. For cultured cells, use 1ml TRIzol solution for 5×10^6 cells.
2. Homogenize the suspension by shaking moderately for several seconds. Incubate 5 min at room temperature (20-30°C).
3. Add 0.2ml chloroform for each 1ml of TRIzol. Shake for 15 seconds, and incubate for additional 2-3 min at room temperature.
4. Spin the samples in a microcentrifuge at 10,000 RPM at 4°C for 15 min.
5. Transfer 550µl of the aqueous phase to an eppendorf tube for each 1ml of TRIzol. (Approximately 600µl can be recovered but for DNA contamination prevention, 550µl is recommendable.)
7. Transfer aqueous phase, add **0.8ml** isopropanol, invert the tubes to mix, and incubate in room temperature for 2-3 min.
9. Spin the tubes in a microcentrifuge at 12,000 RPM at 4°C for 15 min.
10. Take off the supernatant, wash the pellet with 1ml of 70% ethanol, and spin at 12,000 RPM at 4°C for 5 min.
11. Dry the pellet in air for approximately 10 min. Avoid completely drying the pellets as this will decrease the solubility of the RNA.
12. Dissolve the RNA in 50µl of RNase free water, measure its concentration, and keep it in the freezer (–80°C) until further use.