Protocol for RNA Isolation using TRIzol® Reagent with Phase Lock GelHeavy

Phase Lock Gel may be used in conjunction with TRIzol Reagent for the isolation of total RNA from cell and tissue samples. Increased yields are observed using this method, because the entire aqueous phase can be recovered without interphase contamination. Below is a protocol outlining the steps involved in RNA isolation with TRIzol Reagent and Phase Lock Gel-Heavy. For further details about TRIzol Reagent, please refer to TRIzol Reagent protocol.

IMPORTANT: When working with TRIzol Reagent, use appropriate protective clothing and work under a chemical fume hood.

1. Homogenization:

   1) Tissues: Add 1 ml TRIzol Reagent per 50-100 mg tissue and homogenize with a POLYTRON@ homogenizer. For 1-10 mg quantities of tissue, add 0.8 ml TRIzol Reagent and 0.2 ml 1 mg/ml mussel glycogen and homogenize.

   2) Cells grown in a Monolayer: Lyse cells by adding 1 ml TRIzol Reagent per 10 cm2 area of culture dish. Pipette suspension several times to disrupt cells. For 102-104 cells, add 0.8 ml TRIzol Reagent and 0.2 ml 1 mg/ml mussel glycogen and homogenize.

   3) Cells grown in Suspension: Pellet cells by centrifugation. Do not wash cell pellet. Add 1 ml TRIzol Reagent per 5-10 x 106 animal, plant, or yeast cells or 1 x 107 bacterial cells. Resuspend pellet by pipetting to lyse cells. For 102_104 cells, add 0.8 ml TRIzol Reagent and 0.2 ml 1 mg/ml mussel glycogen and homogenize.

2. Pre-spin the appropriate sized Phase Lock Gel-Heavy tubes briefly to collect gel on tube bottoms(1500 x g for 30 seconds is sufficient to collect gel at tube bottoms).
3. Add cell lysate to the tubes containing pre-spun Phase Lock Gel-Heavy and incubate 5 minutes at 15-30°C.
4. Add 0.2 ml chloroform (or chloroform-isoamyl alcohol) per 1 ml TRIzol Reagent initially used. Cap tubes and shake vigorously for 15 seconds. **DO NOT VORTEX!**
5. Centrifuge samples at no more than 12,000 x g for 10 minutes at 2-8°C.
NOTE: 15 ml and 50 ml PLG-H screw-cap tubes should be centrifuged at or below 2000 x g.
6. Examine phasing. Clear, aqueous phase should be entirely atop Phase Lock Gel. The phenolchloro form phase and cloudy interphase should be below Phase Lock Gel layer. If this is not the case, add another 0.2 ml chloroform (or chloroform-isoamyl alcohol) per 1 ml TRIzol Reagent used initially and shake vigorously. Repeat centrifugation and re-examine phasing.
7. Transfer aqueous phase containing RNA to a fresh tube (aqueous phase may be decanted).
8. Precipitate RNA by adding 0.5 mlIsopropyl alcohol per 1 ml TRIzol Reagent used initially. Mix samples by repeated inversion. Allow samples to incubate at 15-30°C for 10 minutes. Centrifuge samples for 10 minutes at no more than 12,000 x g, 2-8°C. RNA pellet should be visible on side and bottom of tube.
9. Decant supernatant. Add 1 ml 75% ethanol per 1 ml TRIzol Reagent used initially to wash the RNA pellet. Mix samples to dislodge pellet, using a vortex if necessary. Centrifuge samples at no more than 7,500 x g for 5 minutes at 2-8°C.
10. Carefully decant supernatant. Briefly air-dry or vacuum-dry the RNA pellet to remove residual ethanol (5-10 minutes). Do not over dry pellet by centrifugation under vacuum as this will make resuspension more difficult. Dissolve RNA pellet in Molecular Biology Grade water, incubating at 55-60°C for 10 minutes to facilitate dissolution.