

RNA Isolation for Gene Expression Microarray or miRNA Microarray Applications

Note : Please Read the Manual Accompanying the Trizol Reagent Carefully. These protocols are adapted from that manual.

Part A Homogenization of adherent cultured cells in Trizol

Note: expand volume accordingly for larger plates

- 1 Wash 35 mM plates of cells 1X with ice-cold PBS buffer.
- 2 Pipette 1 ml of Trizol on to the plate and wash back and forth across the plate.
- 2 Pipette the cell lysate up and down about 10X, washing the lysate across the plate to remove any residual material.
- 3 Transfer homogenate to a microfuge tube at room temperature.

Part B. Trizol RNA Isolation

Note: If RNA recovery is expected to be less than 5 ug, then add 20 ug of Molecular Biology Grade Glycogen prior to further processing.

- 1 Add 0.2 ml chloroform and vortex for 15 sec.
- 2 Incubate mixtures at 15 to 30C for 2-3 min.
- 3 Spin at full speed (12,000 g) in microfuge at 4C (in refrigerator) for 15 min.
- 4 Remove 450-500ul of aqueous phase and transfer to a new tube.

Note: Do not touch or collect material from interphase.

- 5 Add an equal volume (450-500 ul) of isopropyl alcohol to the tube and vortex for 5 seconds.
- 6 Incube them at 15 to 30C for 2-3 min.

Note: Incubate tubes overnight at -20C here if RNA will be used for miRNA analysis.

- 7 Spin at 12,000 g for 10min at 4C.
- 8 Carefully pour off supernatant, while observing that pellet is not lost from bottom of tube.

Note: Supernatant can be poured in to labeled microfuge tube, to prevent accidental loss of pellet.

- 10 Add 500ul of 75% ethanol.
- 11 Vortex to partially resuspend pellet.
- 12 Spin for 2-3 min at RT.
- 13 Pour off ethanol wash while carefully observing that pellet is not lost.

Note: Ethanol wash can be poured in to labeled microfuge tube, to prevent accidental loss of pellet.

- 14 Spin briefly again to collect residual ethanol at the bottom of tube.
- 15 Remove remaining ethanol with P200 pipette, carefully avoiding pellet.
- 14 Lay tubes down on tissue paper with tops open. Let air dry for 5 min.
- 15 Resuspend in minimum of 1 ul Qiagen RNase-free water per 1-5 ug of RNA expected.
- 16 Measure OD at 260 nm and 280 nm of a 1:50 dilution of the RNA sample.

Use 10 mM Tris pH 7.5 as blank and as the diluent, for the OD reading.

- 17 Calculate concentration in ng/ul by multiplying by 40 ug/ml = 1 OD and by the dilution.

For miRNA applications stop here, the remainder of processing will occur at ORB.