**ISOLATION OF TOTAL RNA WITH TRIZOL REAGENT: (Life technologies cat#15596)**

**Reagents Required:**

BCP (1 bromo 3 chloro propane, Isopropyl alcohol 75% ethanol )
Molecular grade water
Centrifuge pre-cooled at 4◦C.
Heating block-able to heat 37◦c, 42◦c, 70◦c
Ice bucket

**RNase away treatment of necessary Instruments and pipettors:**

Spray all pipetters and homogenizer probe with RNase away for 5 minute◦. Rinse thoroughly with distilled water followed by 100% methanol. Dry thoroughly by running Clean the homogenizer probe with 3 changes of molecular grade water and 2 changes of methanol. Run the probe empty for few minutes. Label the falcon tubes(352059) and pipette out 1 ml of tri reagent in each tube.

**Homogenization**

Homogenize 10 to 50 mg of frozen muscle piece in 1ml of tri reagent in falcon tube. Keep all the tubes cold till homogenization is over. Leave the tubes at room temperature for 5 minutes to permit complete dissociation of nucleoprotein complexes. Add 100 ul of BCP. Cap the tube. Vortex it for 10 seconds. Incubate at R.T. for 10 minutes. Centrifuge at full speed for 15 minutes at 4◦C.
Following centrifugation, the mixture separates into 3 layers: Lower red phenol-choloroform phase, middle interphase and colorless upper aqueousphase.

**RNA precipitation**

**Pre-heat the heating block to 55-60◦c**

Leaving some aqueous phase behind, carefully transfer the upper aqueous phase to fresh 1.5 ml tube. Add 0.5ml of isopropanol. Vortex briefly. Incubate at room temperature for 10 min followed by centrifugation at 4◦C for 8 mints. The RNA precipitates can be visualized as a small palette. Carefully remove the supernatant. Wash the palette with 1ml. of 75% ethanol. Mix by brief vortexing and centrifuge at full speed at4◦C for 5 mintes. Discard the supernatant while watching the pallet**.( caution Pallet remains loose in 70% ethanol)**
Air dry the pallet, wipe out the sides with clean swab. Add about 20 ul of mol. Grade water if the starting material is about 10 mg. Heat the tube for 5 mint @60◦C. Mix and spin it down. Heat for another 5mints and spin it down mix contents and spin it down.

**RNA concentration**

Dilute 2ul with 8ul of water. Set up Nanodrop instrument for RNA measurement. Use 2ul of diluted RNA to estimate. Save remaining 8 ul. to check the quality on agarose gel. Freez the sample at -80◦C if Dnase treatment and RT reaction is not planned.

**Dnase1 Treatment (Optional) with DNA-free kit Cat#AM1906 applied Biosystems(Ambion)**

**Total reaction volume 20ul. for 1.5ug total RNA**.
Set up the reaction as follows.
Preheat the block at 37◦C.
First pipette out volume of mol. Grade H2O enough for 1.5ug total RNA so that total volume is 15ul./sample. Keep it cold.

Prepare the master mix as follows;(prepare for 5 tubes extra)
Dnase 1 10x buffer = 2.0ul x no. of tubes
Dnase1 = 1.0ul x no. of tubes
Mol. Grade H2O = 2.0ul x no. of tubes
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5.0ul x no. of tubes

Pipette out 5.0 ul in each of the above reaction tubes. Add 1.5ug RNA per sample. Now the volume becomes 20ul per tube. Keep the reaction tubes cold till all the tubes are finished pipettting. Incubate at 37◦C for 30min.

Add 4.0ul Inactivation reagent/tube. Incubate for 2.0 min**. Mix every 30 to 60 seconds**. This step is very important in order to redisperse the DNase inactivation reagent.
Spin at maximum speed for 2 minutes.