Total RNA preparation from *Drosophila* embryo

Reagents:

Trizol
Chloroform
Isopropyl alcohol
75% Ethanol (in DEPC-treated water)
DEPC-treated water (Ambion)

Procedure:

1. Put 200ul of Trizol reagent in EP tube containing proper number of embryo. Homogenize samples sufficiently. The sample volume should not exceed 10% of the volume of Trizol used for homogenization.
2. Incubate the homogenized samples for 5 minutes at 15 to 30°C to permit the complete dissociation of nucleoprotein complexes.
3. Add 0.2 ml of chloroform per 1 ml of Trizol. Shake tubes vigorously by hand for 15 seconds and incubate them at 15 to 30°C for 2 to 3 minutes.
4. Centrifuge samples at no more than 12,000 × g for 15 minutes at 2 to 8°C. RNA remains exclusively in the colorless aqueous phase.
5. Carefully transfer the aqueous phase into a fresh tube without disturbing the interphase.
6. Precipitate RNA by adding 0.5 ml of isopropyl alcohol per 1 ml of Trizol to each sample. Incubate samples at 15 to 30°C for 10 minutes or -20°C O/N.
7. Centrifuge at no more than 12,000 × g for 10 minutes at 2 to 8°C.
8. Remove the supernatant and wash the RNA pellet once with cold 75% ethanol. Adding at least 1 ml of 75% ethanol per 1 ml of Trizol.
9. Vortex and centrifuge samples at no more than 7,500 × g for 5 minutes at 2 to 8°C.
10. Air-dry or vacuum dry the RNA pellet for 5-10 minutes. Do not dry the RNA by centrifugation under vacuum. It is important not to let the RNA pellet dry completely as this will greatly decrease its solubility.
11. Dissolve RNA in RNase-free water and incubate for 10 minutes at 55 to 60°C.
12. Determine sample concentration and purity by measuring OD at 260nm and 280nm. The A260/A280 ratio should be above 1.6.