Total RNA Isolation from Filarial Parasites Using the Trizol LS Reagent

Before Starting: All reagents should be for RNA use only. EtOH solutions should be made with nuclease free or DEPC treated water. Clean your entire workspace and pipettes with RNase Zap[1]. Wear a clean lab coat and be sure to change gloves frequently!

1. Defrost worms over ice and transfer to a 2 ml round bottom tube. Use of a round bottom tube is important to allow enough space for the BB to vibrate within the tube (step 4).

2. Add 750 μl Trizol LS[2] for every 250 μl of worms in buffer (3:1). Note: Be sure to measure the volume of worms because this ratio is very important.

3. Do 3 freeze/thaw cycles: 3 minutes in Dry Ice/EtOH bath followed by 3 min at 80°C.

4. Add one 3mm stainless steel BB to the 2 ml round bottom tube and attach to vortex with special adaptor.[3] Vortex on the highest speed for 45 minutes, stopping every 10 minutes to change the position of the tube to ensure all areas of the tube are equally mixed.

5. Spin tube briefly before opening and add 200 μl chloroform for every 250 μl of worms in buffer. Vortex briefly and incubate for 3 minutes at room temperature.

6. Transfer the entire sample (except BB) to a pre-spun[4] Phase Lock gel tube[5]. Mix by inversion. **Do NOT Vortex**

7. Centrifuge at 4°C for 15 minutes at 11,900 x g (no more than 12,000 x g).

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[1] Ambion catalog #9780

[2] The Trizol LS reagent (Invitrogen 10296-010) is a monophasic solution of phenol and guanidine isothiocyanate, specially formulated for use with tissues in buffer, therefore you are working with a volume of worms in buffer, not a dry weight of tissues.

[3] We use the Vortex Genie Adaptor (Molecular Bio Laboratories Inc catalog #13000-V1). Alternatively, you can tape the tube on its side to the flat portion of a regular vortex mixer platform.

[4] Prior to use, Pre-Spin the phase lock gel at 12,000- 15,000 x g for 30 seconds.

[5] For isolation of total RNA we will be using a 2 ml heavy Phase Lock gel (5 prime catalog # 2302830). Use of the Phase Lock Gel greatly decreases organic contamination from the aqueous phase.

8. Transfer the aqueous phase to a new 1.5 ml tube being careful to avoid the gel interface.

9. To precipitate the RNA, add 500 µl cold isopropanol (per initial 250 µl of worms in buffer). Vortex briefly and incubate for 10 minutes at room temperature.

10. Centrifuge at 4°C for 30 minutes at 12,200 x g to precipitate the RNA. Note: At this step you should be able to see a small white pellet.

11. Carefully remove supernatant without disturbing the pellet.

12. Wash the pellet with 1 ml cold 75% EtOH by gently rocking the tube back and forth. Centrifuge at 4 °C for 5 minutes at 7,500 x g.

13. Carefully remove supernatant without disturbing the pellet. Spin briefly in nanofuge and remove any remaining supernatant.

14. Invert on kimwipe and air dry for 5-10 min or until there is no visible liquid.

15. Resuspend in 50 µl Nuclease Free water. Flick tube gently to mix.

16. Incubate at 55° C for 10 minutes to ensure complete re-suspension of the pellet. Flick tube gently to mix and then spin briefly in nanofuge.

17. Measure the total RNA concentraion using a Nanodrop spectrophotometer or Aligent bioanalyzer.