Total RNA from *Dirofilaria immitis*, Strain Missouri, Stage L3

Catalog No. NR-42501

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Contributor:

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Manufacturer:

Filariasis Research Reagent Resource Center supported by Contract HHSN272201000030I, NIH-NIAID Animal Models of Infectious Disease Program

Product Description:

NR-42501 is a preparation of total RNA extracted from *Dirofilaria immitis* (*D. immitis*), strain Missouri, third stage larvae (L3). *D. immitis*, strain Missouri was originally obtained from TRS Laboratories in Athens, Georgia, USA.[1]

D. immitis is a filarial nematode that causes cardiopulmonary dirofilariasis in wild and domesticated canines and felines, and is the causative parasite of human pulmonary dirofilariasis.[2] *D. immitis* is commonly known as heartworm disease and transmission relies on mosquitos as a vector. In the case of canines, for which *D. immitis* is best adapted, mosquitos deposit infective third stage larvae (L3) on the skin which penetrate the host. Maturation from stage L3 to L4 occurs between 3 and 12 days post-infection followed by a subsequent molt producing juvenile adult worms, between 50 and 70 days post-infection. The first juvenile adult worms arrive in the pulmonary artery and right ventricle of the heart between 70 and 85 days post-infection and reach sexual maturity approximately 120 days post-infection. Adult females are able to produce and release microfilariae between 6 and 9 months post-infection, which can be taken up by mosquitos during a blood meal.[3]

Material Provided:

Each vial of NR-42501 contains 0.5 µg to 2 µg of DNase-treated RNA in TE buffer (1 mM Tris-HCl, 0.1 mM EDTA, pH ~ 8). The concentration is shown on the Certificate of Analysis. The vial should be centrifuged prior to opening.

Packaging/Storage:

NR-42501 was packaged in RNase/DNase-free plastic vials. The product is provided frozen and should be stored at -80°C or colder upon arrival. Freeze-thaw cycles should be minimized.

Citation:

Acknowledgment for publications should read "The following reagent was provided by the NIH/NIAID Filariasis Research Reagent Resource Center for distribution by BEI Resources, NIAID, NIH: Total RNA from *Dirofilaria immitis*, Strain Missouri, Stage L3, NR-42501."

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

Disclaimers:

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References:

1. Michalski, M. L., et al. "The NIH-NIAID Filariasis Research Reagent Resource Center." PLoS Negl. Trop. Dis. 5 (2011): e1261. PubMed: 22140585.

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2. Morchon R., et al. "Heartworm Disease (*Dirofilaria immitis*) and Their Vectors in Europe – New Distribution Trends." Front. Physiol. 3 (2012): e00196. PubMed: 22701433.
3. Simón, F., et al. "Human and Animal Dirofilariasis: the Emergence of a Zoonotic Mosaic." Clin. Microbiol. Rev. 25 (2012): 507-544. PubMed: 22763636.

Appendix I: Total RNA Isolation from Filarial Parasites

Before Starting: All reagents should be for RNA use only. Ethanol (EtOH) solutions should be made with nuclease free or DEPC treated water. Clean your entire workspace and pipettes with RNase*Zap*® (Ambion ® catalog #9780). 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 (Invitrogen® 10296-010) 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.
   *TRIzol® LS 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. 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 [i.e., Vortex Genie® adapter (Mo Bio Laboratories Inc catalog #13000V1); or the tube can be taped on its side to the flat portion of a regular vortex mixer platform.]. 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 2 mL heavy Phase Lock Gel™ tube [Prior to use, pre-spin the tube at 12,000 to 15,000 x g for 30 seconds (5 prime catalog # 2302830).]. Mix by inversion. **Do NOT Vortex**.
   *Use of the Phase Lock Gel™ greatly decreases organic contamination from the aqueous phase.
7. Centrifuge at 4°C for 15 minutes at 11,900 x g (no more than 12,000 x g).
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 (or equivlanet) and air dry for 5 to 10 minutes 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 concentration using a NanoDrop™ spectrophotometer or Agilent bioanalyzer.

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