Total RNA from *Acanthocheilonema viteae*, Strain FR3, Microfilariae

Catalog No. NR-49201

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For research use only. Not for human use.

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-49201 is a preparation of total RNA extracted from *Acanthocheilonema viteae* (*A. viteae*), strain FR3, microfilariae (see Appendix I for RNA isolation information).

A. viteae (formerly *Dipetalonema viteae*), is a filarial nematode with a life cycle consisting of a soft tick (Argasidae) intermediate host and a rodent definitive host.[1] Infective third-stage larvae are transmitted from a soft tick host to the subcutaneous tissue of a rodent during a blood meal. Filariasis develops within 2-3 months as larvae transition to adult worms and release large numbers of microfilariae in the rodent host bloodstream.[2,3] The life-cycle is complete when microfilariae are taken up during subsequent blood meals by a soft tick and develop into infective third-stage larvae.

A. viteae lacks the *Wolbachia* bacterial endosymbiont, which is found in most human-infective filarial nematodes. *Wolbachia* bacteria have been shown to influence host reproductive systems to improve parasitic advantage.[4]

Material Provided:

Each vial of NR-49201 contains 0.5 µg to 2.0 µg of 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-49201 was packaged in 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 *Acanthocheilonema viteae*, Strain FR3, Microfilariae, NR-49201."

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.

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References:

1. Maki, J. and P. P. Weinstein. "Transplantation into Jirds as a Method of Assessing the Viability and Reproductive Integrity of Adult *Acanthocheilonema viteae* from

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Culture." J. Parasitol. 77 (1991): 749-754. PubMed: 1919923.
2. Pogonka, T., et al. "*Acanthocheilonema viteae*: Characterization of a Molt-Associated Excretory/Secretory 18 kDa Protein." Exp Parasitol. 93 (1999): 73-81. PubMed: 10502469.
3. Michalski, M. L., et al. "The NIH-NIAID Filariasis Research Reagent Resource Center." PLoS Negl. Trop. Dis. 5 (2011): e1261. PubMed: 22140585.
4. Slatko B. E., M. J. Taylor and J. M. Foster. "The *Wolbachia* Endosymbiont as an Anti-Filarial Nematode Target." Symbiosis 51 (2010): 55-65. Pubmed: 20730111.

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 equivalent) 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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