

**Genomic DNA from Adult Female *Dirofilaria immitis*, Strain Missouri 2005**

**Catalog No. NR-42493**

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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-42493 is a preparation of genomic DNA extracted from adult female *Dirofilaria immitis* (*D. immitis*), strain Missouri 2005. *D. immitis*, strain Missouri 2005 was originally obtained from TRS Laboratories in Athens, Georgia, USA.<sup>1</sup>

*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.<sup>2</sup> *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 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.<sup>3</sup>

Humans and felines are much less suitable hosts. In humans, *D. immitis* may be able to reach a branch of the human pulmonary artery, but would trigger an immune response that destroys the immature nematodes; this infrequently results in pulmonary nodules.<sup>3</sup> In felines, cardiopulmonary dirofilariasis follows a similar life cycle as in canines, but is often asymptomatic, and there is a marked reduction in nematode fertility and viability.<sup>3</sup>

**Material Provided:**

Each vial of NR-42493 contains 0.5 µg to 2 µg of RNase A treated genomic DNA 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-42493 was packaged in plastic vials. The product is provided frozen and should be stored at -20°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: Genomic DNA from Adult Female *Dirofilaria immitis*, Strain Missouri 2005, NR-42493."

**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/bmb15/index.htm](http://www.cdc.gov/biosafety/publications/bmb15/index.htm).

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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.
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.

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**Appendix I: Genomic DNA Isolation from Filarial Parasites**

1. Thaw worms over ice and transfer to a 2 mL round bottom tube. Add 100 µL lysis buffer (0.1M EDTA, 0.1M Tris- pH 7.5, and 0.2M NaCl; sterilized by autoclaving) per 250 µL worms in buffer.
2. Add one 5mm BB [5 mm stainless steel scientific beads from Qiagen® (catalog # 69989)] to the 2 mL round bottom tube and attach to vortex mixer with special adaptor [Vortex Genie Adaptor, MO 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.] 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.
3. Centrifuge the tube quickly before opening. Add an additional 150 µL of lysis buffer and vortex briefly. Note: The total volume of lysis buffer should be equal to the starting volume of worms in buffer.
4. Add 30 µL 10% SDS.
5. Add 2 µL of β-mercaptoethanol and vortex briefly.
6. Add 60 µL of Qiagen® Proteinase K (20 mg/ml). Vortex gently. Centrifuge the tube briefly in a nanofuge.
7. Incubate at 65°C for 4 hours to overnight.
8. Add 1.5 µL RNase A (100 mg/mL) and vortex gently. Spin the tube briefly in nanofuge.
9. Incubate at 37°C for 1 hour.
10. Centrifuge the tube briefly. Add 1 volume Tris-buffered phenol (pH 7.9). Vortex to mix.
11. Centrifuge at 2,000 rpm for 5 minutes.
12. Carefully remove the top aqueous phase and transfer to a new 1.5 mL tube.
13. Add an additional 1 volume phenol and vortex to mix.
14. Centrifuge at 2,000 rpm for 5 minutes.
15. Carefully extract aqueous phase and transfer to new 1.5 mL tube.
16. Add 1 volume chisam (24:1 chloroform/isoamyl alcohol). Vortex to mix.
17. Centrifuge at 2,000 rpm for 5 minutes.
18. Carefully remove the top aqueous phase and transfer to a new 1.5 mL tube.
19. Add 1 volume cold isopropanol and 1/10 volume 3M sodium acetate. Vortex Briefly to mix. Note: At This point the DNA extraction can be stored at -20°C. If working with small amounts of starting materials you may add 3-6 µL of glycogen (5 mg/mL) to aid precipitation.
20. Centrifuge at 16,000 x g for 30 minutes at 4°C.
21. Carefully remove isopropanol without disturbing the pellet.
22. Wash the pellet with 1 mL cold ethanol (70-75%).
23. Centrifuge at 16,000 x g for 15 minutes at 4°C.
24. Carefully remove ethanol without disturbing the pellet. Resuspend in 50 µL 0.1X TE or nuclease-free water.
25. If necessary, incubate DNA for 10 minutes at 55°C to completely redissolve pellet.
26. Measure concentration using the nanodrop spectrophotometer. It is also a good idea to run your genomic DNA on a gel to check for the presence of RNA and the integrity of genomic DNA.
27. To remove residual organics dialyze genomic DNA against a beaker (approximately 250 mL) of 0.1X TE for 4 hours to overnight using a Millipore [47mm diameter 0.025 µm filters from Millipore (catalog number VSWP04700)] membrane. Note: If there are no residual organics, this step is not necessary.