

Product Information Sheet for NR-48820

Leishmania tropica, Strain NIH RUP (MHOM/AF/87/RUP)

Catalog No. NR-48820

This reagent is the tangible property of the U.S. Government.

For research use only. Not for use in humans.

Contributor:

David L. Sacks, Ph.D., Chief, Intracellular Parasite Biology Section, Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, Maryland, USA

Manufacturer:

BEI Resources

Product Description:

Protozoa Classification: *Trypanosomatidae*, *Leishmania*

Species: *Leishmania tropica*

Subgenera: *Leishmania*

Strain: NIH RUP (MHOM/AF/87/RUP) (also referred to as MHOM/AF/97/RP)¹

Original Source: *Leishmania tropica* (*L. tropica*), strain NIH RUP (MHOM/AF/87/RUP) was isolated in 1997 from the reactive lesion of a patient with cutaneous leishmaniasis in Afghanistan.^{2,3}

Comments: *L. tropica*, strain NIH RUP (MHOM/AF/87/RUP) has been used to study lipophosphoglycan-mediated midgut attachment in *Phlebotomus sergenti* and has produced cutaneous lesions in Syrian hamsters.^{1,4}

Leishmaniasis is caused by parasitic protozoa of the genus *Leishmania*, which is transmitted to both humans and animals by female phlebotomine sandflies.^{5,6} The sandflies inject the infective stage (promastigotes) of the parasite from their proboscis. Promastigotes that reach the puncture wound are phagocytized by macrophages and other types of mononuclear phagocytic cells. Inside the cells promastigotes transform into the tissue stage of the parasite (amastigotes) and multiply by simple division and infect other mononuclear phagocytic cells. Infection is endemic throughout the tropics, subtropics, and Mediterranean basin.^{5,6}

The current taxonomic classification includes two subgenera, *Leishmania*, which are found in the midgut of the vector's intestine, and *Viannia*, which are found in the hindgut of the vector's intestine. Additionally, the more than 30 known species of *Leishmania* are divided into New World and Old World species, whose divergence is thought to correspond to the separation of the continents millions of years ago. The subgenera *Leishmania* is comprised of New and Old World species while the subgenera *Viannia* is comprised of only New World species.^{7,8} Pathogenic species of both subgenera have also been grouped into complexes based on phylogenetic analyses.⁹

Material Provided:

Each vial of NR-48820 contains approximately 0.5 mL of culture in cryopreservative [5% dimethylsulfoxide (DMSO)].

Please refer to the Certificate of Analysis for the specific culture media used for each lot and to Appendix I for cryopreservation instructions.

Packaging/Storage:

NR-48820 was packaged aseptically in cryovials and is provided frozen on dry ice. The product should be stored at -130°C or colder, preferably in the vapor phase of a liquid nitrogen freezer. If liquid nitrogen storage facilities are not available, frozen cryovials may be stored at -70°C or colder for approximately one week.

Note: Do not under any circumstances store vials at temperatures warmer than -70°C. Storage under these conditions will result in the death of the culture.

To ensure the highest level of viability, the culture should be initiated immediately upon receipt. Any warming of the product during shipping and transfer must be avoided, as this will adversely affect the viability of the product. For transfer between freezers and for shipping, the product may be placed on dry ice for brief periods, although use of a portable liquid nitrogen carrier is preferred. Please read the following recommendations prior to using this material.

Growth Conditions:

Medium 199 (M199) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (HIFBS) and 10 µg/mL hemin

Incubation:

Temperature: 25°C

Atmosphere: Aerobic

Propagation:

1. Place the frozen vial in a 35°C to 37°C water bath and thaw for approximately 2 to 3 minutes. Immerse the vial just enough to cover the frozen material. Do not agitate the vial. Do not leave the vial in the water bath after it is thawed.
2. Immediately after thawing, aseptically transfer the contents of the vial to a T-25 tissue culture flask containing 10 mL M199 medium.
3. Screw the cap on tightly and incubate the tube or flask at 25°C. Observe the culture daily under an inverted microscope for the presence of promastigote forms of the parasite. Subculture when the culture reaches peak density.

Maintenance:

1. When the culture is at or near peak density, transfer approximately 0.1 mL to 0.2 mL into a new flask containing 5 mL to 10 mL fresh M199 medium.
2. Screw the caps on tightly and incubate at 25°C and examine daily under an inverted microscope.
3. Transfer the culture every 2 to 4 days as described in Maintenance steps 1 and 2. The transfer interval will depend on the size of the inoculum and the quality of the medium. This should be determined empirically by examining the culture on a daily basis until conditions for stable growth have been achieved. Do not allow the culture to overgrow. Viability of the culture may be affected soon after reaching peak density.

Please see Appendix I for cryopreservation instructions.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Leishmania tropica*, Strain NIH RUP (MHOM/AF/87/RUP), NR-48820."

Biosafety Level: 2

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 (BMBL). Current Edition. Washington, DC: U.S. Government Printing Office.

Disclaimers:

You are authorized to use this product for research use only. It is not intended for human use.

Use of this product is subject to the terms and conditions of the BEI Resources Material Transfer Agreement (MTA). The MTA is available on our Web site at www.beiresources.org.

While BEI Resources uses reasonable efforts to include accurate and up-to-date information on this product sheet, neither ATCC® nor the U.S. Government makes any warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. Neither ATCC® nor the U.S. Government warrants that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, use and disposal. ATCC® and the U.S. Government are not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, the U.S. Government, ATCC®, their suppliers and contributors to BEI Resources are not liable for damages arising from the misidentification or misrepresentation of products.

Use Restrictions:

This material is distributed for internal research, non-commercial purposes only. This material, its product or its derivatives may not be distributed to third parties. Except as performed under a U.S. Government contract, individuals contemplating commercial use of the material, its products or its derivatives must contact the contributor to determine if a license is required. U.S. Government contractors may need a license before first commercial sale.

References:

1. Kamhawi, S., et al. "The Vectorial Competence of *Phlebotomus sergenti* is Specific for *Leishmania tropica* and is Controlled by Species-Specific, Lipophosphoglycan-Mediated Midgut Attachment." *Parasitology* 121 (2000): 25-33. PubMed: 11085222.
2. Sacks, D. L., Personal Communication.

3. McDowell, M. A., et al. "*Leishmania* Priming of Human Dendritic Cells for CD40 Ligand-Induced Interleukin-12p70 Secretion Is Strain and Species Dependent." *Infect. Immun.* 70 (2002): 3994-4001. PubMed: 12117904.
4. Lira, R., et al. "*Leishmania tropica*: The Identification and Purification of Metacyclic Promastigotes and Use in Establishing Mouse and Hamster Models of Cutaneous and Visceral Disease." *Exp. Parasitol.* 89 (1989): 331-342. PubMed: 9676711.
5. Chappuis, F., et al. "Visceral Leishmaniasis: What Are the Needs for Diagnosis, Treatment and Control?" *Nat. Rev. Microbiol.* 5 (2007): 873-882. PubMed: 17938629.
6. Reithinger, R., et al. "Cutaneous Leishmaniasis." *Lancet Infect. Dis.* 7 (2007): 581-596. PubMed: 17714672.
7. Schönián, G., E. Cupolillo and I. Mauricio. "Molecular Evolution and Phylogeny of *Leishmania*." *Drug Resistance in Leishmania Parasites: Consequences, Molecular Mechanisms and Possible Treatments*. Eds. A. Ponte-Sucre, E. Diaz, and M. Padrón-Nieves. Vienna: Springer, 2013. 15-44.
8. Lainson, R. and J. J. Shaw. "Evolution, Classification and Geographical Distribution." *The Leishmaniasis in Biology and Medicine. Volume I. Biology and Epidemiology*. Eds. W. Peters and R. Killick-Kendrick. London: Academic Press, 1987. 1-120.
9. Schönián, G., et al. "Molecular Epidemiology and Population Genetics in *Leishmania*." *Med. Microbiol. Immunol.* 190 (2001): 61-63. PubMed: 11770112.

ATCC® is a trademark of the American Type Culture Collection.



APPENDIX I: CRYOPRESERVATION

1. To harvest the *Leishmania* culture, remove the media containing promastigotes from infected culture flasks that have reached peak density and transfer to 15 mL plastic centrifuge tubes. Centrifuge at $800 \times g$ for 10 minutes.
2. Remove all but 0.5 mL of the supernatant from each tube, resuspend the cell pellets and pool them into a single tube.
3. Adjust the cell concentration to 4×10^7 to 8×10^7 cells/mL with fresh M199 supplemented with 10% HIFBS and 10 μ g/mL hemin.
Note: If the concentration of cells is too low, centrifuge at $800 \times g$ for 10 minutes and resuspend in a smaller volume of fresh medium to yield the desired parasite concentration.
4. Mix equal volumes of parasite suspension and fresh medium containing 5% DMSO to yield a final concentration of 2×10^7 to 4×10^7 cells/mL in 5% DMSO. Alternatively, glycerol may be used as a cryoprotectant at a final concentration of 10%. The freezing process should start 15 to 30 minutes following the addition of cryoprotective solution to the cell suspension.
Note: To prevent culture contamination, penicillin-streptomycin solution (ATCC® 30-2300™) may be added to a final concentration of 50 IU/mL to 100 IU/mL penicillin and 50 μ g/mL to 100 μ g/mL streptomycin.
5. Dispense 0.5 mL aliquots into 1 mL to 2 mL sterile plastic screw-capped vials for cryopreservation.
6. Place the vials in a controlled rate freezing unit. From room temperature cool the vials at $-1^\circ\text{C}/\text{min}$ to -40°C . If the freezing unit can compensate for the heat of fusion, maintain rate at $-1^\circ\text{C}/\text{min}$ through this phase. At -40°C , plunge vials into liquid nitrogen. Alternatively, place the vials in a Nalgene 1°C freezing container. Place the container at -80°C for 1.5 to 2 hours and then plunge vials into liquid nitrogen.
7. Store in either the vapor or liquid phase of a nitrogen refrigerator (-130°C or colder).