Product Information Sheet for NR-48822

Leishmania donovani, Strain 9515 (MHOM/IN/95/9515)

Catalog No. NR-48822

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For research use only. Not for use in humans.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

Protozoa Classification: *Trypanosomatidae*, *Leishmania*
Species: *Leishmania donovani*
Subgenera: *Leishmania*
Strain: 9515 (MHOM/IN/95/9515)
Original Source: *Leishmania donovani (L. donovani)*, strain 9515 (MHOM/IN/95/9515) was isolated in 1995 from the splenic aspirate of a patient with visceral leishmaniasis in India.[1,2]
Comment: *L. donovani*, strain 9515 (MHOM/IN/95/9515) has been used to study the role of IL-27 in T cell IL-10 regulation in human visceral leishmaniasis.[3]

Leishmaniasis is caused by parasitic protozoa of the genus *Leishmania*, which is transmitted to both humans and animals by female phlebotomine sandflies.[4,5] 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.[4,5]

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.[6,7] Pathogenic species of both subgenera have also been grouped into complexes based on phylogenetic analyses.[8]

Material Provided:

Each vial of NR-48822 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 refer to Appendix I for cryopreservation instructions.

Packaging/Storage:

NR-48822 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 of complete 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 to 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 donovani*, Strain 9515 (MHOM/IN/95/9515), NR-48822."

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:

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References:

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2. 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.
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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 × 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 µg/mL hemin.
   Note: If the concentration of cells is too low, centrifuge at 800 × 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 µg/mL to 100 µg/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°C/min to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C/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).

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