Product Information Sheet for NR-10462

555Acanthamoeba lenticulata, Strain 72/2

Catalog No. NR-10462

For research use only. Not for human use.

Contributor:
Dr. Julia Walochnik, Associate Professor, Department of Parasitology, Medical University of Vienna, Austria.

Manufacturer:
BEI Resources

Product Description:
Protozoa Classification: *Acanthamoebidae*, *Acanthamoeba*
Species: *Acanthamoeba lenticulata*
Strain: 72/2
Original Source: *Acanthamoeba lenticulata* (*A. lenticulata*), strain 72/2 was isolated from the nasal mucosa of a healthy individual in Germany.[1,2]
Comment: *A. lenticulata*, strain 72/2 was deposited to BEI Resources as type T5, morphology group III, based on 18S ribosomal RNA gene sequence analysis.[1,2]

A. lenticulata isolates are the most divergent of the group III strains with some isolates containing group I introns, suggesting a common ancestor.[2] Though T5 isolates are the second most abundant environmental clade, the genotype represents the minority of reported keratitis cases, with the majority of strains causing keratitis belonging to sequence type 4.[3,4]

Amoebae belonging to the genus *Acanthamoeba* inhabit a wide variety of environmental niches worldwide and have been isolated from soil, freshwater, air, humans, and animals, both domestic and feral, and are able to exist both as free-living amoebae and as parasitic pathogens.[4] In healthy humans, *Acanthamoeba* is the causative agent of *Acanthamoeba* keratitis, an increasingly-prevalent sight-threatening eye disease among contact lens wearers. In immunocompromised individuals, *Acanthamoeba* can cause disseminated infections of other tissues and, in severe cases, the fatal disease granulomatous amebic encephalitis.[4-6]

Acanthamoeba are currently classified by twelve sequence types (T1 to T12) based on nuclear small ribosomal subunit RNA genotyping and divided into three morphological groups: Group I (T7, T8, T9), Group II (T3, T4, T11) and Group III (T1, T2, T5, T6, T10, T12).[4] Identification of *Acanthamoeba* on the genus level is based on spiny surface projections (acanthopodia) present on the surface of trophozoites.[4] Highly-specific PCR methods for subgeneric identification of isolates have been developed for both clinical and environmental applications.[7]

Material Provided:
Each vial of NR-10462 contains approximately 0.5 mL of culture in cryopreservative. Please see Appendix I for cryopreservation instructions.

Packaging/Storage:
NR-10462 was packaged aseptically in screw-capped plastic cryovials and is provided frozen on dry ice. The product should be stored at cryogenic temperature (-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 insure 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:
ATCC® medium 997: Fresh water amoeba agar medium inoculated with *Enterobacter aerogenes (E. aerogenes)* (ATCC® 13048™)
Note: *A. lenticulata* feeds on microorganisms and must be grown in the presence of a feeder layer.
Incubation:
Temperature: 20°C to 25°C
Atmosphere: Aerobic
Propagation:
1. Prior to initiating the culture of NR-10462, streak an ATCC medium 997 agar plate with *Enterobacter aerogenes* (ATCC® 13048™) and incubate at 35°C to 37°C overnight.
2. Place the frozen vial of NR-10462 in a 35°C to 37°C water bath and thaw for approximately 2 to 3 minutes. Do not agitate the vial. Do not leave the vial in the water bath after it is thawed.
3. Immediately after thawing, aseptically transfer the contents of the vial to the agar plate inoculated with *E. aerogenes*. Distribute the material evenly over the plate using a spread bar.
4. Wrap the entire edge of the plate with parafilm and incubate upright at 20°C to 25°C. Trophozoites should be observed within 5 to 7 days.

Maintenance:
1. Remove an agar block (~ 5 mm^2) with trophozoites from the edge of the agar plate and invert the block at the edge of a new plate previously inoculated with *E. aerogenes*.
2. Wrap the entire edge of the plate with parafilm and incubate upright at 20°C to 25°C.
3. Repeat steps 1 and 2 every 10 to 14 days.

Citation:
Acknowledgment for publications should read "The following reagent was obtained through the BEI Resources, NIAID,

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NIH: *Acanthamoeba lenticulata*, Strain 72/2, NR-10462."

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. 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. J. Walochnik, personal communication.
2. Stothard, D. R., et al. "The Evoluntionary History of the Genus *Acanthamoeba* and the Identification of Eight New 18S rRNA Gene Sequence Types." J. Eukaryot. Microbiol. 45 (1998): 45-54. PubMed: 9495032.
3. Ledee, D. R., et al. "Molecular Identification of T4 and T5 Genotypes in Isolates from *Acanthamoeba* Keratitis Patients." J. Clin. Microbiol. 47 (2009): 1458-1462. PubMed: 19321730.
4. Marciano-Cabral, F. and G. Cabral. "*Acanthamoeba* spp. as Agents of Disease in Humans." Clin. Microbiol. Rev. 16 (2003): 273-307. PubMed: 12692099.
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6. Clarke, D. W. and J. Y. Niederkorn. "The Pathophysiology of *Acanthamoeba* Keratitis." Trends Parasitol. 4 (2006): 175-180. PubMed: 16500148.
7. Schroeder, J. M., et al. "Use of Subgenic 18S Ribosomal DNA PCR and Sequencing for Genus and Genotype Identification of Acanthamoebae from Humans with Keratitis and from Sewage Sludge." J. Clin. Microbiol. 39 (2001): 1903-1911. PubMed: 11326011.

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E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

APPENDIX I: CRYOPRESERVATION

1. Harvest *Acanthamoeba* from multiple agar plates by adding 5 mL of Dryl's Solution (ATCC® medium 5080) to each plate and rubbing the surface of the agar with a spreader to detach adhering trophozoites.
2. Transfer the cell suspensions to 15 mL or 50 mL plastic centrifuge tubes.
3. Adjust the cell concentration to 1.0 to 4.0 x 10^6 cells/mL with fresh Dryl's Solution.
   Note: If the concentration of cells is too low, centrifuge at 1300 x g for 10 minutes and resuspend in a smaller volume of fresh medium to yield the desired cell concentration.
4. Mix equal volumes of cell suspension and fresh medium containing 15% DMSO to yield a final concentration of 0.5 to 2.0 x 10^6 cells/mL in 7.5% DMSO. The freezing process should start 15 to 30 minutes following the addition of cryoprotective solution to the cell suspension.
5. Dispense 0.5 mL aliquots into 1 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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