Product Information Sheet for NR-33656

Acanthamoeba sp., Strain CDC:V026

Catalog No. NR-33656

For research use only. Not for human use.

Contributor:
Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Manufacturer:
BEI Resources

Product Description:
Protozoa Classification: *Acanthamoebidae*, *Acanthamoeba*
Species: *Acanthamoeba* sp.
Strain: CDC:V026
Original Source: *Acanthamoeba* sp., strain CDC:V026 was isolated from the contact lens of a woman in Louisiana.[1]
Comment: *Acanthamoeba* sp., strain CDC:V026 was deposited to BEI Resources as type T4, based on 18S ribosomal RNA gene sequence analysis.[1]

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.[2] 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.[2-5]

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).[2] Identification of *Acanthamoeba* on the genus level is based on spiny surface projections (acanthopodia) present on the surface of trophozoites.[2] Highly-specific PCR methods for subgeneric identification of isolates have been developed for both clinical and environmental applications.[6]

Material Provided:
Each vial of NR-33656 contains approximately 0.5 mL of culture in cryopreservative. Please see Appendix I for cryopreservation instructions.

Packaging/Storage:
NR-33655 was packaged aseptically in screw-capped plastic 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 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:
Peptone Yeast Glucose (PYG) medium (ATCC® medium 712) (Appendix II)
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. 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 5 to 10 mL ATCC® medium 712.
3. Screw the cap on tightly and incubate the tube or flask at 25°C.

Maintenance:
1. When the culture is at or near peak density, vigorously agitate or scrape the surface of the flask to detach adherent cells.
2. Transfer approximately 0.25 mL to a fresh flask containing 5 to 10 mL fresh ATCC® medium 712.
3. Screw the caps on tightly and incubate at 25°C.
4. The amoeba will form an almost continuous sheet of cells on the bottom surface of the flask. Repeat steps 1 through 3 every 7 to 10 days.

Please refer to Appendix I for cryopreservation instructions.

Citation:
Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Acanthamoeba* sp., Strain CDC:V026, NR-33656."

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

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References:

1. Sriram, R., et al. "Survival of Acanthamoeba Cysts After Dessication for More Than 20 Years." J. Clin. Microbiol. 46 (2008): 4045-4048. PubMed: 18923013.
2. Marciano-Cabral, F. and G. Cabral. "*Acanthamoeba* spp. as Agents of Disease in Humans." Clin. Microbiol. Rev. 16 (2003): 273-307. PubMed: 12692099.
3. Visvesvara, G. S. "Amebic Meningoencephalitides and Keratitis: Challenges in Diagnosis and Treatment." Curr. Opin. Infect. Dis. 23 (2010): 590-594. PubMed: 20802332.
4. Clarke, D. W. and J. Y. Niederkorn. "The Pathophysiology of *Acanthamoeba* Keratitis." Trends Parasitol. 4 (2006): 175-180. PubMed: 16500148.
5. Walochnik, J., et al. "Discrimination between Clinically Relevant and Nonrelevant *Acanthamoeba* Strains Isolated from Contact Lens-Wearing Keratitis Patients in Austria." J. Clin. Microbiol. 38 (2000): 3932-3936. PubMed: 11060047.
6. 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.

Support Provided by NIAID

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APPENDIX I: CRYOPRESERVATION

1. To harvest the *Trypanosoma* culture, remove the media containing trypanosomes from infected culture flasks that have reached peak density and transfer to 15 mL plastic centrifuge tubes. Centrifuge at 800 × g for 10 min.
2. Remove all but 0.5 mL of the supernatant from each tube, resuspend the cell pellets, and pool them to a single tube.
3. Adjust the cell concentration to 0.5×10^7 to 1×10^7 cells/mL with fresh growth medium.
   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 20% dimethylsulfoxide (DMSO) or glycerol to yield a final concentration of 2.5×10^6 to 5×10^6 cells/mL in 10% DMSO or glycerol. 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 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).

APPENDIX II: TRYPANOSOME MEDIUM (ATCC® MEDIUM 431)

Solid Phase:

Beef extract	3.0 g
Peptone	5.0 g
NaCl	8.0 g
Agar	15.0 g
Distilled water	1.0 L

Liquid Phase (Locke's solution):

NaCl	8.0 g
KCl	0.2 g
$CaCl_2$	0.2 g
KH_2PO_4	0.3 g
Glucose	2.5 g
Distilled water	1.0 L

Adjust pH of both phases to 7.2 to 7.4. Autoclave both phases at 121°C for 15 minutes. Cool the solid phase mixture to about 45°C and aseptically add 30% sterile, defibrinated, rabbit blood. Aseptically dispense in sterile 16 × 125 mm screw-capped test tubes in 5 mL amounts and cool on a slant. After cooling the slants, aseptically dispense 3.0 mL of Locke's solution (Liquid phase) over each slant.

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