Encephalitozoon cuniculi, CDC:V282

Catalog No. NR-9703

(Derived from ATCC® 50602™)

For research use only. Not for human use.

Contributor:

Centers for Disease Control and Prevention, Atlanta, Georgia

Manufacturer:

Biodefense and Emerging Infections Research Resources Repository

Product Description:

Fungal Classification: *Unikaryonidae, Encephalitozoon*
Species: *Encephalitozoon cuniculi*
Strain/Isolate: CDC:V282
Original Source:[1,2] Urine from AIDS patient, Colorado, 1993
Comment: NR-9703 was derived from ATCC® 50602™, which was deposited to ATCC® by G. S. Visvesvara of the CDC. *Encephalitozoon cuniculi,* CDC:V282 is genotype III based on PCR analyses of ITS (internal transcribed spacer of the rRNA gene), PTP (polar tube protein) and SWP-1 (spore wall protein-1).[2]

Encephalitozoon cuniculi (*E. cuniculi*) is an obligate, amitochondriate intracellular parasite. It can infect a variety of mammals including rabbits, rats, mice, horses, foxes, cats, dogs, muskrats, leopards, baboons and humans. Three genotypes were characterized based on sequence repeats found in the ITS of the rRNA gene. Genotype I was identified in rabbit isolates, genotype II in mice and genotype III in dogs. The human isolates that have been genotyped are usually type I or III.[2] Infection due to *E. cuniculi* occurs through contact with their spores that contain a polar tubule, which is tightly coiled within the spore. The tubule is extruded upon interaction with a suitable host cell and the sporoplasm is transferred. *E. cuniculi* has been identified as an opportunistic pathogen of immunocompromised humans, especially AIDS patients. Currently, a number of isolates of *E. cuniculi* originating from different human specimens, including urine, bronchoalveolar lavage, sputum and brain, have been established in culture.[3]

Material Provided:

Each vial of NR-9703 contains approximately 0.5 mL of culture in cryopreservative. Please see Appendix I below for cryopreservation instructions.

Packaging/Storage:

NR-9703 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:

Growth Media and Host Cells:
Eagle's Minimum Essential Medium (EMEM) supplemented with 3% heat inactivated fetal bovine serum.
African green monkey kidney epithelial cells (BS-C-1; ATCC® CCL-26™). Other cell lines that support growth of *E. cuniculi* include human lung fibroblasts (WI-38; ATCC® CCL-75™) and Madin-Darby canine kidney (MDCK) epithelial cells (ATCC® CCL-34™).[3]
Incubation:
Temperature: 35°C to 37°C
Atmosphere: 95% air, 5% CO_2
Propagation:

1. To establish a culture from the frozen state, place a vial in a 35 to 37°C water bath. Thawing time is 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 into a tissue culture flask containing a fresh monolayer of African green monkey kidney epithelial cells and EMEM containing 3% (v/v) HIFBS.
3. Outgas the flask for 10 seconds with a 95% air, 5% CO_2 gas mixture.
4. Incubate in a 35 to 37°C CO_2 incubator with the caps screwed on tightly. Observe the culture daily under an inverted microscope for the presence of spores growing inside parasitophorous vacuoles.

Maintenance:

1. Remove the medium from a fresh confluent monolayer of African green monkey kidney epithelial cells in a tissue culture flask and replace it with 10 mL of medium containing 3% (v/v) HIFBS.
2. Remove the medium from the *Encephalitozoon cuniculi* culture when approximately 50% of the African green monkey kidney epithelial cell monolayer has lysed. Centrifuge the spores that had been released into the medium at 1300 x g for 10 minutes.
3. Remove the supernatant and resuspend the cell pellet in a small volume (0.5 to 1.0 mL) of EMEM containing 3% (v/v) HIFBS or phosphate buffered saline (PBS). Transfer the resuspended pellet to the fresh flask of African green monkey kidney epithelial cells, prepared in step 1 above. Follow steps 3 and 4 in Propagation.

© 2008/2011 American Type Culture Collection (ATCC). All rights reserved.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: *Encephalitozoon cuniculi,* CDC:V282, NR-9703."

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, 2007; see www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm.

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 make 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. De Groote, M. A., et al. "Polymerase Chain Reaction and Culture Confirmation of Disseminated *Encephalitozoon cuniculi* in a Patient with AIDS: Successful Therapy with Albendazole." J. Infect. Dis. 171 (1995): 1375-1378. PubMed: 7751721.
2. Xiao, L., et al. "Genotyping *Encephalitozoon cuniculi* by Multilocus Analyses of Genes with Repetitive Sequences." J. Clin. Microbiol. 39 (2001): 2248-2253. PubMed: 11376065.
3. Visvesvara, G. S. "*In Vitro* Cultivation of Microsporidia of Clinical Importance." Clin. Microbiol. Rev. 15 (2002): 401-413. PubMed: 12097248.
4. Katinka, M. D., et al. "Genome Sequence and Gene Compaction of the Eukaryote Parasite *Encephalitozoon cuniculi*." Nature 414 (2001): 450-453. PubMed: 11719806.
5. Didier, E. S., et al. "Identification and Characterization of Three *Encephalitozoon cuniculi* Strains." Parasitology 111 (1995): 411-421. PubMed: 11023405.
6. Peuval, I., et al. "Polymorphism of the Gene Encoding a Major Polar Tube Protein PTP1 in Two Microsporidia of the Genus *Encephalitozoon*." Parasitology 121 (2000): 581-587. PubMed: 11155928.

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

Biodefense and Emerging Infections Research Resources Repository
P.O. Box 4137
Manassas, VA 20108-4137 USA
www.beiresources.org
800-359-7370
Fax: 703-365-2898
E-mail: contact@beiresources.org

© 2008/2011 American Type Culture Collection (ATCC). All rights reserved.

APPENDIX I: CRYOPRESERVATION

1. To harvest the *Encephalitozoon cuniculi* culture, detach any remaining tissue culture cells (infected and uninfected) by scraping the surface of the flask with a cell scraper.
2. Transfer the cell suspension (including parasites) to 15 mL plastic centrifuge tubes. Centrifuge at 1300 x g for 10 min.
3. Remove all but 0.5 mL of the supernatant from each tube, resuspend the cell pellets, and pool them to a single tube.
4. Pass the resulting cell suspension through a syringe equipped with a 27-gauge ½-inch needle to break up any remaining cells.
5. Adjust the parasite concentration to 2.0 to 4.0 x 10^7 cells/mL with fresh medium [Eagle's Minimum Essential Medium (EMEM) supplemented with 3% heat inactivated fetal bovine serum or Dulbecco's PBS (ATCC® 30-2200) can be used].
   Note: If the concentration of parasites is too low, centrifuge at 1300 x g for 10 min and resuspend in a smaller volume of fresh medium to yield the desired parasite concentration.
6. Mix equal volumes of parasite suspension and fresh medium or PBS containing 20% DMSO and 6% HIFBS to yield a final concentration of 1.0 to 2.0 x 10^7 cells/mL in 10% DMSO, 3% HIFBS. The freezing process should start 15 to 30 minutes following the addition of cryoprotective solution to the parasite suspension.
   Note: To prevent culture contamination, penicillin-streptomycin solution (ATCC® 30-2300) may be added to a final concentration of 50 to 100 U/mL penicillin and 50 to 100 µg/mL streptomycin.
7. Dispense 0.5 mL aliquots into 1 to 2 mL sterile plastic screw-capped vials for cryopreservation.
8. 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.
9. Store in either the vapor or liquid phase of a nitrogen refrigerator (-130°C or colder).

Biodefense and Emerging Infections Research Resources Repository
P.O. Box 4137
Manassas, VA 20108-4137 USA
www.beiresources.org

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

© 2008/2011 American Type Culture Collection (ATCC). All rights reserved.