

***Encephalitozoon cuniculi*, Strain CDC:V282**

Catalog No. NR-9703

(Derived from ATCC® 50602™)

For research use only. Not for use in humans.

Contributor:

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

Manufacturer:

BEI Resources

Product Description:

Classification: *Unikaryonidae, Encephalitozoon*

Species: *Encephalitozoon cuniculi*

Strain/Isolate: CDC:V282

Original Source: *Encephalitozoon cuniculi* (*E. cuniculi*), strain CDC:V282 was isolated in 1993 from the urine of an AIDS patient in Colorado, USA.^{1,2}

Comments: NR-9703 was derived from ATCC® 50602™, which was deposited to ATCC® by G. S. Visvesvara of the CDC. *E. cuniculi*, strain CDC:V282 was deposited as genotype III based on PCR analyses of internal transcribed spacer (ITS) of the ribosomal RNA gene, polar tube protein (PIP) and spore wall protein-1 (SWP-1).²

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 internal transcribed spacer (ITS) of the ribosomal RNA 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 (10% glycerol). 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-9703 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 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:

Growth Media and Host Cells:

Eagle's Minimum Essential Medium (EMEM) modified to contain Earle's Balanced Salt Solution, non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate and 1500 mg/L sodium bicarbonate (ATCC® 30-2003™) supplemented with 10% heat-inactivated fetal bovine serum (HIFBS)

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 epithelial cells (MDCK; ATCC® CCL-34™).³

Incubation:

Temperature: 35°C to 37°C

Atmosphere: Aerobic with 5% CO₂

Propagation:

1. To establish a culture from the frozen state, place a vial in a 35°C 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 10% (v/v) HIFBS.
3. Outgas the flask for 10 seconds with a 95% air, 5% CO₂ gas mixture.
4. Incubate in a 35°C to 37°C CO₂ 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 10% (v/v) HIFBS.
2. Remove the medium from the *E. cuniculi* culture when approximately 50% of the African green monkey kidney cell monolayer has lysed. Centrifuge the spores that had been released into the medium at 1300 × g for 10 minutes.
3. Remove the supernatant and resuspend the cell pellet in a small volume (0.5 mL to 1 mL) of EMEM containing 10% (v/v) HIFBS or phosphate buffered saline (PBS). Transfer

the resuspended pellet to the fresh flask of African green monkey kidney cells prepared in step 1 above. Follow steps 3 and 4 in Propagation.

Please refer to Appendix I for cryopreservation instructions.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Encephalitozoon cuniculi*, Strain 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 \(BMBL\)](#). 6th ed. Washington, DC: U.S. Government Printing Office, 2020.

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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. Peupal, 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.

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APPENDIX I: CRYOPRESERVATION

1. To harvest the *E. cuniculi* culture, detach any remaining cells (infected and uninfected) by scraping the surface of the flask with a cell scraper and transfer to 15 mL plastic centrifuge tubes. Centrifuge at $1300 \times g$ for 10 min.
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. Pass the resulting cell suspension through a syringe equipped with a 27-gauge ½-inch needle to break up any remaining cells.
4. Adjust the parasite concentration to 2×10^7 to 4×10^7 cells/mL using fresh growth medium.
Note: If the concentration of parasites is too low, centrifuge at $1300 \times g$ for 10 min and resuspend in a smaller volume of fresh medium or PBS to yield the desired parasite concentration.
5. Mix equal volumes of parasite suspension and fresh medium or PBS containing 20% dimethylsulfoxide (DMSO) and 20% HIFBS to yield a final concentration of 1×10^7 to 2×10^7 cells/mL in 10% DMSO, 10% 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 IU/mL to 100 IU/mL penicillin and 50 µg/mL to 100 µg/mL streptomycin.
6. Dispense 0.5 mL aliquots into 1 mL to 2 mL sterile plastic screw-capped vials for cryopreservation.
7. 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.
8. Store in either the vapor or liquid phase of a nitrogen refrigerator (-130°C or colder).