Encephalitozoon cuniculi, Strain Type 2

Catalog No. NR-20773

For research use only. Not for human use.

NR-20773 is contaminated with *Mycoplasma* sp. Please determine whether or not this product is acceptable for your intended use.

Contributor:

Louis M. Weiss, Department of Medicine and Pathology, Albert Einstein College of Medicine, Bronx, NY

Manufacturer:

BEI Resources

Product Description:

Fungal Classification: *Unikaryonidae, Encephalitozoon*
Species: *Encephalitozoon cuniculi*
Strain: Type 2
Original Source: *Encephalitozoon cuniculi* (*E. cuniculi*), strain type 2 was derived from a laboratory mouse circa 1972.[1,2]
Comment: *E. cuniculi,* strain type 2 is contaminated with *Mycoplasma.*

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.[3] 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.[4] 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.[5]

Material Provided:

Each vial of NR-20773 contains approximately 0.5 mL of culture in cryopreservative. Please see Appendix I below for cryopreservation instructions.

Packaging/Storage:

NR-20773 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) (ATCC® 30-2003™) supplemented with 10% heat inactivated fetal bovine serum (HIFBS).
Rabbit kidney epithelial cells (RK13; ATCC® CCL-37™)
Other cell lines that support growth of *E. cuniculi* include African green monkey kidney epithelial cells (BS-C-1; ATCC® CCL-26™) and Madin-Darby canine kidney (MDCK) epithelial cells (ATCC® CCL-34™) and human lung fibroblasts (WI-38; ATCC® CCL-75™).[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°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 rabbit kidney epithelial cells and EMEM containing 10% (v/v) HIFBS.
4. Incubate in a 35°C to 37°C in a 95% air, 5% CO_2 atmosphere. 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 rabbit 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 rabbit 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 10% (v/v) HIFBS or phosphate buffered saline (PBS). Transfer the resuspended pellet to the fresh flask of rabbit kidney epithelial cells, prepared in step 1 above. Follow steps 3 and 4 in Propagation.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Encephalitozoon cuniculi,* Strain Type 2, NR-20773."

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 http://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:

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BEI Resources
www.beiresources.org

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

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) (ATCC® 30-2003) 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 20% HIFBS to yield a final concentration of 1.0 to 2.0 x 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 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).

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Tel: 800-359-7370
Fax: 703-365-2898