Product Information Sheet for NR-9701

Encephalitozoon hellem, Strain CDC:0291:V213

Catalog No. NR-9701

(Derived from ATCC® 50451™)

For research use only. Not for use in humans.

Contributor:

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

Manufacturer:

BEI Resources

Product Description:

Protozoa Classification: *Unikaryonidae*, *Encephalitozoon*
Species: *Encephalitozoon hellem*
Strain: CDC:0291:V213
Original Source: *Encephalitozoon hellem (E. hellem)*, CDC:0291:V213 was isolated in 1991 from the urine of an adult human male AIDS patient in Georgia, USA.[1]
Comments: *E. hellem*, CDC:0291:V213 was derived from ATCC® 50451™, which was deposited to ATCC® by G. S. Visvesvara of the CDC.

E. hellem is a primitive spore-forming Gram-positive eukaryotic microsporidian that lacks mitochondria and is a cause of disease in both animals and humans.[1] Some *E. hellem* isolates have been identified as opportunistic pathogens of humans, especially AIDS patients. *E. hellem* has been isolated from various clinical specimens such as corneal scrapings, urine, broncho-alveolar lavage, sputum and nasal mucosa.[2]

Material Provided:

Each vial of NR-9701 contains approximately 0.5 mL of culture in cryopreservative [10% dimethylsulfoxide (DMSO)]. Please refer to Appendix I for cryopreservation instructions.

Packaging/Storage:

NR-9701 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:

Eagle's Minimal Essential Medium (EMEM), adjusted to contain 10% (v/v) heat-inactivated fetal bovine serum (HIFBS)
Human lung fibroblasts (WI-38; ATCC® CCL-75™)
Other cell lines that support growth of *E. hellem* include African green monkey kidney epithelial cells (BS-C-1; ATCC® CCL-26™), Madin-Darby canine kidney (MDCK) epithelial cells (ATCC® CCL-34™) and rabbit kidney epithelial cells (RK13; ATCC® CCL-37™).[3]
Incubation:
Temperature: 35°C to 37°C
Atmosphere: Aerobic with 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 to a tissue culture flask containing a fresh monolayer of human lung fibroblast cells (ATCC® CCL-75™) and 10 mL of EMEM containing 10% (v/v) HIFBS.
3. Outgas the flask for 10 seconds with a 95% air, 5% CO_2 gas mixture.
4. Incubate in a 35°C 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 host cell monolayer in a tissue culture flask and replace it with 10 mL of EMEM containing 10% (v/v) HIFBS.
2. Remove the medium from the *E. hellem* culture when approximately 50% of the host 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 1.0 mL) of growth medium or phosphate buffered saline (PBS). Transfer the resuspended pellet to the fresh culture flask 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 hellem*, Strain CDC:0291:V213, NR-9701."

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. 6th ed. Washington, DC: U.S. Government Printing Office, 2020; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

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References:
1. Visvesvara, G. S., et al. "Culture, Electron Microscopy, and Immunoblot studies on a Microsporidian Parasite Isolated from the Urine of a Patient with AIDS." J. Protozool. 38 (1991): 105S-111S. PubMed: 1818126.
2. Didier, E. S. and L. M. Weiss. "Microsporidiosis: Current Status." Curr. Opin. Infect. Dis. 19 (2006): 485-492. PubMed: 16940873.
3. Visvesvara, G. S. "*in vitro* Cultivation of Microsporidia of Clinical Importance." Clin. Microbiol. Rev. 15 (2002): 401-413. PubMed: 12097248.

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Support Provided by NIAID

APPENDIX I: CRYOPRESERVATION

1. To harvest the *Encephalitozoon hellem* 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 × 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 per mL using fresh growth medium.
   Note: If the concentration of parasites is too low, centrifuge at 1300 × 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 per 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 per mL to 100 IU per mL penicillin and 50 100 µg per mL to 100 µg per 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°C per min to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C per 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).

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