Encephalitozoon intestinalis, Alveolar Isolate

Catalog No. NR-9702

(Derived from ATCC® 50506™)

For research use only. Not for use in humans.

Contributor:

Elizabeth S. Didier, Division of Microbiology, Tulane National Primate Research Center, Covington, Louisiana, USA

Manufacturer:

BEI Resources

Product Description:

Classification: *Unikaryonidae, Encephalitozoon*

Species: *Encephalitozoon intestinalis* (formerly *Septata intestinalis*)[1]

Isolate: Alveolar

Original Source: *Encephalitozoon intestinalis (E. intestinalis)*, Alveolar isolate was obtained in 1994 from the bronchoalveolar lavage specimen of an AIDS patient in New York, New York, USA.[2]

Comments: NR-9702 was derived from ATCC® 50506™, which was deposited to ATCC® by E. S. Didier. The complete genome of *E. intestinalis*, Alveolar isolate has been sequenced (GenBank: GCA_024399295).

Microsporidia are common unicellular obligate intracellular parasites, which infect a wide range of invertebrate and vertebrate hosts. *E. intestinalis* is the second most common microsporidian that infects immunocompromised humans, especially AIDS patients. *E. intestinalis* was initially associated with chronic diarrhea but was later found to spread intravascularly to the kidneys, lungs and nasal sinuses.[2]

Material Provided:

Each vial of NR-9702 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-9702 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)

Human lung fibroblasts (WI-38; ATCC® CCL-75™)

Other cell lines that support growth of *E. intestinalis* include African green monkey kidney epithelial cells (BS-C-1; ATCC® CCL-26™) and Madin-Darby canine kidney epithelial cells (MDCK; ATCC® CCL-34™).[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 into a tissue culture flask containing a fresh monolayer of human lung fibroblast cells and 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 monolayer of human lung fibroblast 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. intestinalis* culture when approximately 50% of the human lung fibroblast 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 human lung fibroblast 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 intestinalis*, Alveolar Isolate, NR-9702."

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.

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 makes 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. Hartskeerl, R. A., et al. "Genetic and Immunological Characterization of the Microsporidian *Septata intestinalis* Cali, Kotler and Orenstein, 1993: Reclassification to *Encephalitozoon intestinalis*." Parasitology 110 (1995): 277-285. PubMed: 7724235.
2. Didier, E. S., et al. "Characterization of *Encephalitozoon* (*Septata*) *intestinalis* Isolates Cultured from Nasal Mucosa and Bronchoalveolar Lavage Fluids of Two AIDS Patients." J. Eukaryot. Microbiol. 43 (1996): 34-43. PubMed: 8563708.
3. Visvesvara, G. S. "*In Vitro* Cultivation of Microsporidia of Clinical Importance." Clin. Microbiol. Rev. 15 (2002): 401-413. PubMed: 12097248.

Support Provided by NIAID

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 *E. intestinalis* 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/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/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°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.
8. Store in either the vapor or liquid phase of a nitrogen refrigerator (-130°C or colder).

BEI Resources
www.beiresources.org

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