To establish a cell culture from the frozen state place an ampule in a water bath set at 35°C (2-3 min). Immediately after thawing, aseptically transfer contents to a T-25 tissue culture flask containing a minimum of 30 ml Minimum Essential Medium (EMEM) with 2 mM L-glutamine and Earle's BSS adjusted to pH 7.4 with HEPES. Incubate in a 35°C CO2 incubator with the caps screwed on tightly. When the cell line forms a confluent layer, remove all the medium and replace it with 2 mL of 0.25% trypsin dissolved in Hank's Balanced Salt Solution. Gently distribute the trypsin over the monolayer, remove the trypsin, and place the flask at 35°C for 10 min. Outgas the flask for 10 seconds with a 95% air, 5% CO2 gas mixture. Incubate in a 35°C CO2 incubator with the caps screwed on tightly.

Storage and Culture Initiation
Frozen ampules packed in dry ice should either be thawed immediately or stored in liquid nitrogen. If liquid nitrogen storage facilities are not available, frozen ampules may be stored at or below -70°C for approximately one week. Do not under any circumstance store frozen ampules at refrigerator freezer temperatures (generally -20°C). Storage of frozen material at this temperature will result in the death of the culture.

1. To thaw a frozen ampule, place it in a 35°C water bath such that the lip of the ampule remains above the water line. Thawing time is approximately 2 to 3 minutes. Do not agitate the ampule. Do not leave ampule in water bath after it is thawed.
2. Immediately after thawing, aseptically transfer contents to a T-25 tissue culture flask containing a fresh monolayer of ATCC® CCL-26™ cells and 10 mL ATCC® 30-2003 with 3% (v/v) HIFBS.

Citation of Strain
If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: *Encephalitozoon hellem* (ATCC® 50451™)
3. Outgas the flask for 10 seconds with a 95% air, 5% CO₂ gas mixture.
4. Incubate in a 35°C CO₂ incubator with the caps screwed on tightly.

Culture Maintenance
1. Remove the medium from a fresh confluent monolayer of CCL-26™ cells in a T-25 tissue culture flask and replace it with 10 mL of ATCC® 30-2003 with 3% (v/v) HIFBS.
2. To transfer the culture, remove the old medium containing the organism and centrifuge at 1300 x g for 10 min.
3. Remove the supernatant and resuspend the cell pellet. Transfer the resuspended pellet to the fresh flask of CCL-26™ cells.
4. Outgas the flask for 10 seconds with a 95% air, 5% CO₂ gas mixture.
5. Incubate in a 35°C CO₂ incubator with the caps screwed on tightly.

Cryopreservation

Harvest and Preservation
1. Harvest the culture by gently agitating the contents of each flask. Transfer all but approximately 1 mL of the culture medium to 15 mL plastic centrifuge tubes. Detach the remaining tissue culture cells (infected and uninfected) by scraping the surface of the flask with a cell scraper. Pass the resulting cell suspension through a syringe equipped with a 27 gauge 1/2 in needle and pool this suspension with the culture fluid.
2. Spin the cell suspensions at approximately 50 x g for 3 min, to remove the cellular debris.
3. Transfer the spore suspensions (supernatants) to new 15 mL plastic centrifuge tubes. Centrifuge at 1300 x g for 10 min.
4. Pool the spore pellets and adjust the concentration to 2.0 - 4.0 x 10⁷ cells/mL with a fresh solution of Hank's Balanced Salt Solution. If the concentration is too low centrifuge at 1300 x g for 10 min and resuspend in the volume of Hank's Balanced Salt Solution required to yield the desired concentration.
5. Mix the spore preparation and 20% (v/v) DMSO in equal portions. The final concentration will be 1.0 - 2.0 x 10⁷ cells/mL and 10% DMSO. The time from the mixing of the cell preparation and the cryoprotective solution before the freezing process begins should be no less than 15 min. and no more than 30 min.
6. Dispense in 0.5 mL aliquots to 1.0-2.0 mL sterile plastic screw-capped cryules (special plastic vials for cryopreservation).
7. Place the vials in a controlled rate freezing unit. From room temperature cool 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 the heat of fusion. At -40°C plunge into liquid nitrogen. Alternatively, place the vials in a Nalgene 1°C freezing apparatus. Place the apparatus at -80°C for 1.5 to 2 hours and then plunge ampules into liquid nitrogen. (The cooling rate in this apparatus is approximately -1°C/min.)
8. Store in either the vapor or liquid phase of a nitrogen refrigerator.
9. To thaw a frozen ampule, place it in a 35°C water bath such that the lip of the ampule remains above the water line. Thawing time is approximately 2 to 3 minutes. Do not agitate the ampule. Do not leave ampule in water bath after thawed.
10. Immediately after thawing, aseptically transfer contents to a T-25 tissue culture flask containing a fresh monolayer of ATCC® CCL-26™ cells and 10 mL ATCC® 30-2003 with 3% (v/v) HIFBS.
11. Outgas the flask for 10 seconds with a 95% air, 5% CO₂ gas mixture.
12. Incubate in a 35°C CO₂ incubator with the caps screwed on tightly.

References
References and other information relating to this product are available online at www.atcc.org.

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the Biosafety in Microbiological and Biomedical Laboratories from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media
may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant 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, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.

Additional information on this culture is available on the ATCC web site at www.atcc.org.

Please read this FIRST

**Storage Temp.**

*Frozen Cultures:*

-70°C for 1 week;

liquid N₂ vapor

for long term storage

*Freeze-dried Cultures:*

2-8°C

*Live Cultures:*

See Protocols section for handling information

**Biosafety Level**

2

**Intended Use**

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

**Citation of Strain**

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: *Encephalitozoon hellem* (ATCC® 50451™)