ATCC

Product Sheet

Encephalitozoon hellem (ATCC® 50504™)

Please read this FIRST

Storage Temp.

Frozen Cultures:
-70°C for 1 week; liquid N_2 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® 50504™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Deposited Name: *Encephalitozoon hellem* Didier et al.
Depositor: ES Didier
Isolation: Corneal scrapings (?) from HIV-seropositive human with microsporidial keratoconjunctivitis, New York City, NY, 1990

Propagation

Growth Conditions
Temperature: 35°C
Atmosphere: 5% CO_2
Cell Line: ATCC® CCL-75™ (lung, human), ATCC® CCL-34™ (kidney, canine), or ATCC® CCL-26™ (kidney, African green monkey).

Instructions for Complete Medium
Eagle's Minimum Essential Medium (EMEM) (ATCC® cat. 30-2003) with 2 mM L-glutamine and Earle's BSS adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, and 1.0 mM sodium pyruvate; supplemented with 3% fetal bovine serum (ATCC® cat. 30-2020).

Protocols

Cell Line Maintenance

1. To establish a cell culture from the frozen state place an ampule in a water bath set at 35°C (2-3 min). Immerse the vial just sufficient to cover the frozen material. Do not agitate the vial.
2. Immediately after thawing, aseptically remove the contents of the ampule and inoculate into 10.0 mL of fresh ATCC® 30-2003 with 10% (v/v) Heat-Inactivated Fetal Bovine Serum (HIFBS)* in a T-25 tissue culture flask.
3. Outgas the flask for 10 seconds with a 95% air, 5% CO_2 gas mixture.
4. Incubate in a 35°C CO_2 incubator with the cap screwed on tightly.
5. Change the medium 1-2 times per week.
   *Fetal bovine serum is available from ATCC (catalog number 30-2020). Serum is heat-inactivated by exposure to 56°C for 30 minutes. This treatment will inactivate proteins of the complement pathway. Remove the serum from the refrigerator and aseptically distribute in 100 mL aliquots to sterile 125 mL screw-capped bottles. Immerse bottles in a 35°C water bath for 5 minutes. Do not directly transfer bottles from the refrigerator to 56°C. Transfer the bottles to a 56°C water bath and begin timing for 30 minutes. To avoid contamination, do not allow the level of the water in the bath to come in contact with the lip of the screw cap. It is best to leave one inch between the serum level in the bottle and the lip of the cap and to fill the water bath to a level just slightly above the level of the serum. To assure even heating of the serum, swirl the bottle(s) every ten minutes. **Note:** Some suppliers provide serum already heat-inactivated.

Transferring the Cell Line

1. When the cell line forms a confluent layer, remove all the medium and replace it with 3 mL of Phosphate Buffered Saline (PBS) (ATCC® cat. 30-2200). Incubate T-25 flask at 35°C for 25-30 min.
2. Remove all the PBS and replace it with 2 mL of 0.25% (w/v) trypsin dissolved in Hank's Balanced Salt Solution (ATCC® cat. 30-2101).
3. Gently distribute the trypsin over the monolayer, remove the trypsin, and place the flask at 35°C for 10 min.
4. Add 2 mL of ATCC® 30-2003 with 10% (v/v) HIFBS and detach any cells still adherent by alternately aspirating the medium into a pipette and discharging the contents over the monolayer.
5. Distribute the cell suspension in 0.5 mL aliquots to 4 T-25 flasks containing 10 mL fresh ATCC® 30-2003 with 10% (v/v) HIFBS.
6. Outgas the flask for 10 seconds with a 95% air, 5% CO_2 gas mixture.
7. Incubate in a 35°C CO_2 incubator with the cap screwed on tightly.

Storage and Culture Initation
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 ampoules 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.

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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 cells (ATCC® CCL-75™, CCL-34™, or CCL-26™) and 10 mL ATCC® 30-2003 with 3% (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 CO_2 incubator with the cap screwed on tightly.

Culture Maintenance

1. Remove the medium from a fresh confluent monolayer of cells (ATCC® CCL-75™, CCL-34™, or CCL-26™) 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 *Encephalitozoon* culture, remove the old medium containing the organism and centrifuge at 1300 x g for 10 min.
3. Remove all but 0.5 mL of the supernatant and resuspend the cell pellet. Transfer the resuspended pellet to the fresh flask of cells (ATCC® CCL-75™, CCL-34™, or CCL-26™).
4. Outgas the flask for 10 seconds with a 95% air, 5% CO_2 gas mixture.
5. Incubate in a 35°C CO_2 incubator with the cap screwed on tightly.

Cryopreservation

Harvest and Preservation

1. To harvest the *Encephalitozoon* 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. 3Remove 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 1/2 in needle to break up any remaining cells. Adjust the parasite concentration to 2.0 - 4.0 x 10^7 cells/mL with fresh medium or PBS.
   NOTE: If the concentration of parasites is too low, centrifuge at 1300 x g for 10 min and resuspend in the volume of fresh medium or PBS required to yield the desired concentration.
5. Prepare a cryoprotective solution containing 20% (v/v) DMSO and 6% (v/v) HIFBS in fresh medium or PBS.
6. Mix the cell preparation and cryoprotective solution in equal portions. The final concentration will be 1.0 - 2.0 x 10^7 cells/mL, 10% DMSO, and 3% HIFBS. The time from the mixing of the cell preparation and cryoprotective solution to the start of the freezing process should be no less than 15 min. and no more than 30 min.
   NOTE: To prevent culture contamination, penicillin-streptomycin solution (ATCC® 30-2300) may be added to a final concentration of 50 to 100 I.U./mL penicillin and 50 to 100 µg/mL streptomycin.
7. Dispense in 0.5 mL aliquots to 1.0-2.0 mL sterile plastic screw-capped cryules (special plastic vials for cryopreservation).
8. Place vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. If freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through heat of fusion. At -40°C plunge ampules 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.)
9. Store frozen ampules in either the vapor or liquid phase of a nitrogen refrigerator.
10. 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.
11. Immediately after thawing, aseptically transfer contents to a T-25 tissue culture flask containing a fresh monolayer of cells (ATCC® CCL-75™, CCL-34™, or CCL-26™) and 10 mL ATCC® 30-2003 with 3% (v/v) HIFBS.
12. Outgas the flask for 10 seconds with a 95% air, 5% CO_2 gas mixture.
13. Incubate in a 35°C CO_2 incubator with the cap screwed on tightly.

References

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

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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.

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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.

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