



Product Sheet

Encephalitozoon hellem (ATCC® 50504™)

Please read this FIRST



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™)

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: 37.0°C

Duration: 5% CO₂

Protocol: ATCCNO: 50502 SPEC: Thaw ampule in a 37C water bath and aseptically transfer the contents to a T-25 tissue culture flask containing a monolayer of MDCK cells (ATCC CCL-34). Twice a week, remove the culture fluid and feed the infected cells with fresh medium. Centrifuge the culture fluid at 400-500 x g for 10 minutes, discard the supernatant, resuspend the cell pellet in fresh medium and transfer it to a fresh monolayer of host cells. Alternatively, transfer the culture fluid to a 15-ml plastic centrifuge tube and store at 4C in an upright position. The spores can be concentrated simply by allowing them to settle to the bottom of the tube. Remove the supernatant from the tube when the spores have settled. Using this technique the spores can be concentrated to high densities over a period of several months.

Medium

ATCC® Medium 2313: RPMI 1640 medium

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ATCC® Medium 722: Minimum essential medium (MEM)

Cryopreservation

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. 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 1/2 in needle to break up any remaining cells. Adjust the parasite concentration to 2.0 - 4.0 x 10⁷ 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 10⁷ 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% CO₂ gas mixture.
13. Incubate in a 35°C CO₂ incubator with the cap screwed on tightly.

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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.

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Disclaimers

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

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

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