Encephalitozoon cuniculi Levaditi et al.

50503™

Description

Strain designation: lagomorph subtype species
Deposited As: *Encephalitozoon cuniculi* Levaditi et al.
Type strain: No

Storage Conditions

Product format: Frozen
Storage conditions: -80°C or colder for 1 week, vapor phase of liquid nitrogen for long-term storage

Intended Use

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

BSL 2

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Host: WI-38 (ATCC CCL-75); MDCK (NBL-2) (ATCC CCL-34); BS-C-1 (ATCC CCL-26)
Instructions for complete medium: 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
Temperature: 35°C
Atmosphere: 95% Air, 5% CO_2

Handling Procedures

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; contact ATCC Sales to order). 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.

Establishing an *Encephalitozoon* Culture from the Frozen State

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.

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:

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 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.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Encephalitozoon cuniculi* Levaditi et al. (ATCC 50503)

References

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

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Revision

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