



# ***Encephalitozoon cuniculi*** **Levaditi et al.**

**PRA-336™**

## **Description**

**Strain designation:** type 2

**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](http://www.atcc.org).

---

## Growth Conditions

**Host:** RK13 (ATCC CCL-37); MDCK (NBL-2) (ATCC CCL-34)

**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 10% fetal bovine serum

**Temperature:** 35-37°C

---

## 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 sufficiently 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<sup>®</sup> 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<sub>2</sub> gas mixture.
4. Incubate in a 35-37°C CO<sub>2</sub> 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<sup>®</sup> cat. 30-2200). Incubate T-25 flask at 35-37°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<sup>®</sup> cat. 30-2101).
3. Gently distribute the trypsin over the monolayer, remove the trypsin, and place the flask at 35-37°C for 10 min.
4. Add 2 mL of ATCC<sup>®</sup> 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<sup>®</sup> 30-2003 with 10% (v/v) HIFBS.
6. Outgas the flask for 10 seconds with a 95% air, 5% CO<sub>2</sub> gas mixture.
7. Incubate in a 35-37°C CO<sub>2</sub> incubator with the cap 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 ampoules may be stored at or below -70°C for approximately one week. **Do not under any circumstance store frozen ampoules 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-37™ or CCL-34™) and 10 mL ATCC® 30-2003 with 10% (v/v) HIFBS.
3. Outgas the flask for 10 seconds with a 95% air, 5% CO<sub>2</sub> gas mixture.
4. Incubate in a 35-37°C CO<sub>2</sub> incubator with the cap screwed on tightly.

**Culture maintenance:**

1. Remove the medium from a fresh confluent monolayer of cells (ATCC® CCL-37™ or CCL-34™) in a T-25 tissue culture flask and replace it with 10 ml of ATCC® 30-2003 with 10% (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-37™ or CCL-34™).
4. Outgas the flask for 10 seconds with a 95% air, 5% CO<sub>2</sub> gas mixture.
5. Incubate in a 35-37°C CO<sub>2</sub> 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  $2.0 - 4.0 \times 10^7$  cells/mL with fresh medium or PBS.

NOTE: If the concentration of parasites is too low, centrifuge at  $1300 \times g$  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 20% (v/v) HIFBS in fresh medium or PBS.
6. Mix the cell preparation and cryoprotective solution in equal portions. The final concentration will be  $1.0 - 2.0 \times 10^7$  cells/mL, 10% DMSO, and 10% 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<sup>®</sup> 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  $-1^\circ\text{C}/\text{min}$  to  $-40^\circ\text{C}$ . If freezing unit can compensate for the heat of fusion, maintain rate at  $-1^\circ\text{C}/\text{min}$  through heat of fusion. At  $-40^\circ\text{C}$  plunge ampules into liquid nitrogen. Alternatively, place the vials in a Nalgene  $1^\circ\text{C}$  freezing apparatus. Place the apparatus at  $-80^\circ\text{C}$  for 1.5 to 2 hours and then plunge ampules into liquid nitrogen. (The cooling rate in this apparatus is approximately  $-1^\circ\text{C}/\text{min}$ .)
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  $35^\circ\text{C}$  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<sup>®</sup> CCL-37<sup>™</sup> or CCL-34<sup>™</sup>) and 10 mL ATCC<sup>®</sup> 30-2003 with 10% (v/v) HIFBS.
12. Outgas the flask for 10 seconds with a 95% air, 5%  $\text{CO}_2$  gas mixture.
13. Incubate in a  $35-37^\circ\text{C}$   $\text{CO}_2$  incubator with the cap screwed on tightly.

---

## Notes

This strain has been found to be *Mycoplasma* positive.

---

## 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 PRA-336)

---

## References

References and other information relating to this material are available at [www.atcc.org](http://www.atcc.org).

---

## Warranty

The product is provided 'AS IS' and the viability of ATCC® products is warranted for 30 days from the date of shipment, provided that the customer has stored and handled the product according to the information included on the product information sheet, website, and Certificate of Analysis. For living cultures, ATCC lists the media formulation and reagents that have been found to be effective for the product. While other unspecified media and reagents may also produce satisfactory results, a change in the ATCC and/or depositor-recommended protocols may affect the recovery, growth, and/or function of the product. If an alternative medium formulation or reagent is used, the ATCC warranty for viability is no longer valid. Except as expressly set forth herein, no other warranties of any kind are provided, express or implied, including, but not limited to, any implied warranties of merchantability, fitness for a particular purpose, manufacture according to cGMP standards, typicality, safety, accuracy, and/or noninfringement.

---

## Disclaimers

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. Any proposed commercial use is prohibited without a [license from ATCC](#).

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 or complete and the customer bears the sole responsibility of confirming the accuracy and completeness of any such information.

This product is sent on the condition that the customer is responsible for and assumes all risk and responsibility in connection with the receipt, handling, storage, disposal, and use of the ATCC product including without limitation taking all appropriate safety and handling precautions to minimize health or environmental risk. As a condition of receiving the material, the customer agrees that any activity undertaken with the ATCC product and any progeny or modifications will be conducted in compliance with all applicable laws, regulations, and guidelines. This product is provided 'AS IS' with no representations or warranties whatsoever except as expressly set forth herein and in no event shall ATCC, its parents, subsidiaries, directors, officers, agents, employees, assigns, successors, and affiliates be liable for indirect, special, incidental, or consequential damages of any kind in connection with or arising out of the customer's use of the product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of such materials.

Please see the material transfer agreement (MTA) for further details regarding the use of this product. The MTA is available at [www.atcc.org](http://www.atcc.org).

---

## Copyright and Trademark Information

© ATCC 2023. All rights reserved.

ATCC is a registered trademark of the American Type Culture Collection.

---

## Revision

This information on this document was last updated on 2024-10-26

---

## **Contact Information**

ATCC

10801 University Boulevard

Manassas, VA 20110-2209

USA

US telephone: 800-638-6597

Worldwide telephone: +1-703-365-2700

Email: [tech@atcc.org](mailto:tech@atcc.org) or contact your local distributor

---