



# ***Perkinsus marinus*** **(Mackin et al.) Levine**

**50757™**

## **Description**

**Strain designation:** HCedar2

**Deposited As:** *Perkinsus marinus* (Mackin et al.) Levine

**Type strain:** No

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## **Storage Conditions**

**Product format:** Frozen

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

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## **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.

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## **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.

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## 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).

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## Growth Conditions

### Medium:

ATCC Medium 1886: Perkinsus broth medium

**Temperature:** 25°C

**Culture system:** Axenic

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## Handling Procedures

### 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**

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**-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, until thawed (2-3 min). Immerse the ampule just enough to cover the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically transfer contents to a T-25 tissue culture flask containing 10 ml of ATCC Medium 1886. Incubate at 25°C.

**Culture maintenance:**

1. Vigorously agitate a culture at or near peak density and aseptically transfer a 0.1 ml aliquot to a T-25 tissue culture flask containing 10 ml of fresh complete medium.
2. Screw cap on tightly and incubate at 25°C.
3. Subculture every 10-14d.

**Cryopreservation:**

1. Harvest cells from several cultures which are in logarithmic to late stationary phase of growth. Vigorously agitate to suspend the cells.
2. Aseptically transfer the cell suspension to 15 ml plastic centrifuge tubes.
3. Centrifuge at 200 x g for 5 min.
4. While cells are centrifuging, prepare a 20% solution of DMSO in ATCC Medium 1886.
5. Remove the supernatant and pool the cell pellets into a final volume of 4.5 ml.
6. Combine the cell suspension with an equal volume of 20% DMSO cryoprotectant solution (prepared in step 4) to yield a final concentration of 10% DMSO.
7. Dispense in 0.5 ml aliquots to 1.0-2.0 ml Nunc vials (special plastic vials for cryopreservation).
8. Place the vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. At -40°C, plunge ampules into liquid nitrogen.
9. Store ampules in a liquid nitrogen refrigerator until needed.
10. To establish a culture from the frozen state, place a frozen ampule in a 35°C water bath just enough to cover the frozen material. Allow the ampule to thaw completely (2-3 min).
11. Immediately after thawing, aseptically remove the contents and transfer to a T-25 tissue culture flask containing 10 ml of fresh ATCC medium 1886.
12. Screw the cap on tightly and incubate at 25°C. Observe the culture daily.

Transfer the culture when many trophozoites are observed.

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## Notes

Additional information on this culture is available on the ATCC web site at [www.atcc.org](http://www.atcc.org).

While every 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.

ATCC recommends that individuals contemplating commercial use of any culture first contact the originating investigator to negotiate an agreement. Third party distribution of this culture is discouraged, since this practice has resulted in the unintentional spreading of contaminated cultures.

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## Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Perkinsus marinus* (Mackin et al.) Levine (ATCC 50757)

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## References

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

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