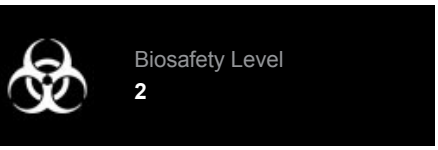
ATCC

Product Sheet

Perkinsus marinus (ATCC® 50509™)

Please read this FIRST



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: *Perkinsus marinus* (ATCC® 50509™)

Description

Strain Designation: DBNJ-1 [NJ-1]
Deposited Name: *Perkinsus marinus* (Mackin et al.) Levine
Depositor: D Bushek
Isolation:
eastern oyster, Crassostrea virginica, Delaware Bay, NJ, 1993

Propagation

Growth Conditions
Temperature: 25.0°C
Duration: axenic
Protocol: ATCCNO: 50439 SPEC: When the frozen ampule arrives, place it directly into a 35C-water bath and transfer its thawed contents to 14 ml of fresh medium in a T-75 tissue culture flask. Maintain by removing 13 ml of cell suspension weekly and replacing with an equal volume of fresh medium. Alternately, aseptically transfer 1.0 ml of a growing culture to 13 ml of fresh medium in a T-75 tissue culture flask. Please note: This strain has wide tolerances to most environmental variables, i.e., temperature range: 15-35C, salinity range: 10-60 parts per thousand; pH range: 6.0-8.5. The distribution of the species worldwide is not known. In order to avert the introduction of this pathogen into non-endemic areas, all culture wastes must be treated as biohazardous and autoclaved prior to disposal. There are no known mechanisms for eradication of this pathogen from the environment.

Medium
ATCC® Medium 1886: Perkinsus broth medium

Instructions for Complete Medium
ATCC Medium 1886

Protocols

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 -20°C).** Storage of frozen material at this temperature will result in the death of the culture.
1. To thaw a frozen ampule, place in a 35°C water bath, until thawed (2-3 min). Immerse the ampule just sufficient to cover the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically transfer the entire contents to a T25 culture flask containing 10 ml of ATCC medium 1886.
3. Screw the cap on tightly and incubate at 25°C.

Culture Maintenance
1. When the culture is at or near peak density, vigorously agitate the culture.
2. Transfer approximately 0.25 ml to a fresh flask containing 10 ml of fresh ATCC medium 1886.
3. Screw the caps on tightly and incubate at 25°C.
4. Repeat steps 1-3 at 10-14 day intervals.

Cryopreservation

1. To achieve the best results set up cultures with several different inocula (e.g. 0.25 ml, 0.5 ml, 1.0 ml). Harvest cultures and pool when the culture that received the lowest inoculum is at or near peak density.
2. If the cell concentration exceeds the required level do not centrifuge, but adjust the concentration to between 2 x 10^6 and 2 x 10^7 cells/ml with fresh growth medium. If the concentration is too low, centrifuge at 600 x g for 5 min and resuspend the pellet in the volume of fresh medium required to yield the desired concentration.
3. While cells are centrifuging prepare a 20% (v/v) solution of sterile DMSO as follows: Add the required volume of DMSO to a glass screw-capped test tube and place it in an ice bath. Allow the DMSO to solidify. Add the required volume of refrigerated medium. Dissolve the DMSO by inverting the tube several times.
*NOTE: If the DMSO solution is not prepared on ice, an exothermic reaction will occur that may precipitate certain components of the medium.

American Type Culture Collection
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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

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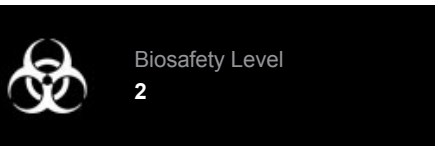

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4. Mix the cell preparation and the DMSO in equal portions. Thus, the final concentration will be between 10^6 and 10^7 cells/ml and 10.0% (v/v) DMSO. The time from the mixing of the cell preparation and DMSO stock solution to the start of the freezing process should be no less than 15 min and no longer than 30 min.
5. Dispense in 0.5 ml aliquots into 1.0 - 2.0 ml sterile plastic screw-capped cryules (special plastic vials for cryopreservation).
6. Place the vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through the heat of fusion. At -40°C plunge 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.)
7. The frozen preparations are stored in either the vapor or liquid phase of a nitrogen freezer.
8. To establish a 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.
9. Immediately after thawing, aseptically remove the contents of the ampule and inoculate into 10 ml of fresh ATCC medium 1886 in a T-25 tissue culture flask. Incubate at 25°C.

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.

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.

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.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable 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.

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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