



Product Sheet

# *Perkinsus marinus* (ATCC®) PRA-240™)

Please read this **FIRST**

Storage Temp.  
**Frozen Cultures:**  
-70°C for 1 week;  
liquid N<sub>2</sub> vapor  
for long term  
storage



**Freeze-dried  
Cultures:**  
2-8°C

**Live Cultures:**  
See Protocols  
section for  
handling  
information



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: *Perkinsus marinus* (ATCC® PRA-240™)

American Type Culture Collection  
PO Box 1549  
Manassas, VA 20108 USA  
[www.atcc.org](http://www.atcc.org)

800.638.6597 or 703.365.2700  
Fax: 703.365.2750  
Email: [Tech@atcc.org](mailto:Tech@atcc.org)

Or contact your local distributor

## Description

**Strain Designation:** CB5D4  
**Depositor:** GR Vasta  
**Isolation:** Atlantic oyster, *Crassostrea virginica*, Chesapeake Bay, MD, USA

## Propagation

**Growth Conditions**  
**Temperature:** 25°C  
**Culture System:** Axenic

**Medium**  
ATCC® Medium 2860: Modified DMEM/F12 Medium

## Instructions for Complete Medium

**Media:** ATCC Medium 2860 is completed with 5% heat-inactivated fetal bovine serum (HIFBS) and 1% lipid concentrate (Sigma L5146 or equivalent).

## Protocols

### 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 -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 T-25 culture flask containing 10 mL of ATCC medium 2860.
3. Screw the cap on tightly and incubate at 25-28°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 2860.
3. Screw the caps on tightly and incubate at 20-25°C.
4. Repeat steps 1-3 at 10-14 day intervals.

## Cryopreservation

### Harvest and Preservation

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  $2 \times 10^6$  and  $2 \times 10^7$  cysts/mL with fresh growth medium. If the concentration is too low, centrifuge at  $600 \times g$  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.
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 before the freezing process is begun 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).



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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 2860 in a T-25 tissue culture flask. Incubate at 25-28°C.



## References

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

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## Disclaimers

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

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