



# ***Perkinsus chesapeaki*** **McLaughlin et al.**

**PRA-425™**

## **Description**

**Strain designation:** 130820 MBWAt-10.1/A5

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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

ATCC Medium 2684: DME/F12-3 Growth Medium (29ppt)

**Instructions for complete medium:** ATCC Medium 1886 and ATCC Medium 2684 are completed just prior to use with 2% and 3% heat-inactivated fetal bovine serum, respectively; both formulations are supplemented with 1% lipid concentrate prior to use. More robust growth may be obtained by

**Temperature:** 25°C

**Atmosphere:** Aerobic

**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  $-70^{\circ}\text{C}$  for approximately one week. **Do not under any circumstance store frozen ampules at refrigerator freezer temperatures (generally  $-20^{\circ}\text{C}$ ).** 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  $35^{\circ}\text{C}$  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 it is thawed.
2. Immediately after thawing, aseptically transfer the entire contents to a T-25 flask containing 10 mL complete medium.
3. Incubate with the cap tightly sealed at  $20\text{--}25^{\circ}\text{C}$ .

**Culture maintenance:** Subculture at peak density (approximately every 10-14d) to a fresh T-25 flask of complete medium in the following manner:

1. Vigorously agitate the flask and aseptically transfer 0.25 mL to a T-25 tissue culture flask containing 10 mL complete medium.
2. Incubate with the cap tightly sealed at  $20^{\circ}\text{C}$  to  $25^{\circ}\text{C}$ .

#### **Reagents for cryopreservation:**

##### **Reagents**

##### Cryoprotective Solution

DMSO 2.0 mL

Fresh complete growth medium 8.0 mL

##### **Cryopreservation:**

##### **Harvest and Preservation**

1. To achieve the best results, set up cultures with several different inocula (i.e., 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  $2 \times 10^7$  cells/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 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  $-1^{\circ}\text{C}/\text{min}$  to  $-40^{\circ}\text{C}$ . If the freezing unit can compensate for the heat of fusion, maintain rate at  $-1^{\circ}\text{C}/\text{min}$  through the heat of fusion. At  $-40^{\circ}\text{C}$  plunge 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  $-1^{\circ}\text{C}/\text{min}$ .)
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  $35^{\circ}\text{C}$  water bath (2-3 min). Immerse the vial just sufficiently 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 complete medium in a T-25 flask. Incubate with the cap tightly sealed at  $20^{\circ}\text{C}$  to  $25^{\circ}\text{C}$ .
10. Follow the protocol for maintenance of culture.

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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 chesapeaki* McLaughlin et al. (ATCC PRA-425)

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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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