Neoparamoeba aestuarina (Page)

0806™

Description

- **Strain designation** Patuxent
- **Deposited As** *Neoparamoeba aestuarina* (Page) Page
- **Type strain** No

Storage Conditions

- **Product format** Test tube
- **Storage conditions** See handling procedure

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 1

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.

Growth Conditions

- **Medium**
  ATCC Medium 1667: Half-strength seawater 802 medium
- **Instructions for complete medium** Media: ATCC Medium 1667 inoculated with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™)
- **Temperature** 25°C

Handling Procedures

- **Handling of Live Culture**
  This strain is routinely shipped as a growing culture in a glass 16 x 125 mm screw-capped test tube. The volume of the cell suspension is approximately 5 mL. When the culture arrives remove it promptly from the shipping container. Do not store the culture at refrigeration temperatures before handling. Immediately place the tube on a 15-degree slant at 25°C. Allow the culture to remain undisturbed for at least three hours before observing it with an inverted microscope. Attached trophozoites should be evident. If their numbers are low the culture may have been exposed to temperature extremes in transit. Regardless of the state of the culture, vigorously agitate the culture and aseptically transfer a 0.5 mL aliquot to a T-25 tissue culture flask containing 10 mL of ATCC medium 1667 bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™). Incubate the parent and daughter cultures at 25°C with the caps tightly sealed (the test tube should be kept on a 15° slant).
- **Culture maintenance** Subculture every two weeks to a fresh T-25 flask of bacterized medium in the following manner:
  1. Vigorously agitate the flask and aseptically transfer 0.5 mL from a growing culture to a T-25 tissue culture flask containing 10 mL of ATCC medium 1667 bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™).
  2. Incubate flask at 25°C with the cap on tightly.
- **Reagents for cryopreservation Reagents**
  Cryoprotective Solution

DMSO, 1.8 mL
Fresh growth medium w/o bacteria , 8.2 mL

- **Cryopreservation**
  1. Mix the components in the order listed. When the medium is added to the DMSO the solution will warm up due to chemical heat.
  2. Harvest cells from a culture that is at or near peak density by filtration and centrifugation at 800 x g for 5 min.
  3. Adjust the concentration of cells at least 2 x 10^6/mL in fresh medium.
  4. Mix the cell preparation and the cryoprotective solution in equal portions.
  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 vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. If freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through heat of fusion. At -40°C plunge ampules 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. Ampules are stored in either the vapor or liquid phase of a nitrogen refrigerator.
  8. To establish a culture from the frozen state place the vial in a 35°C water bath. Immerse the vial to a level just above the surface of the frozen material. Do not agitate the vial. Immediately after thawing, do not leave in water bath, aseptically remove the contents of the ampule and inoculate into a T-25 tissue culture flask containing 10 mL ATCC medium 1667 bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™).
  9. Incubate at 25°C with the cap screwed on tightly.
  10. Once the culture is established, vigorously agitate the flask and aseptically transfer 0.5 mL to 10 mL of bacterized ATCC medium 1667.
  11. Follow the protocol for maintenance of culture.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Neoparamoeba aestuarina* (Page) Page (ATCC 50806)

References

References and other information relating to this material are available at www.atcc.org.

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