Neoparamoeba pemaquidensis (Page) Page

30735™

Description

Strain designation: CH-27
Deposited As: *Paramoeba pemaquidensis* Page
Type strain: No

Storage Conditions

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

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 994: Marine ameba medium
Instructions for complete medium:

Media: ATCC Medium 994

Special Instructions: This culture is bacterized.

Temperature: 25°C
Culture system: Xenic
Incubation: grown with Oceanospirillum sp.

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 ampoules 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 it in a 35°C water bath, until thawed (2-3 min). Immerse the ampule enough to cover only the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically transfer contents to a plate of ATCC medium 994 and distribute evenly over the surface with a spread bar.
3. Seal the plate with Parafilm, place upright in a moist-chamber, and incubate at 25°C. Trophozoites should be seen within 2-3 d.

Culture maintenance:

1. Streak an ATCC medium 994 plate with *Klebsiella pneumoniae* (ATCC® 700831) and incubate at 35°C overnight.
2. Remove an agar block (~5 mm^2) with trophozoites from the edge of an agar plate culture and invert the block at the edge of the freshly bacterized plate.
3. Wrap the entire edge of the plate with Parafilm, place upright in a moist-chamber, and incubate at 25°C.
4. Repeat steps 1-3 at 10-14 d intervals.

Note: a monoxenic amoeba culture can be established in this manner using any suitable bacterial food source.

Cryopreservation:

1. To detach trophozoites from the plate, flush the surface with 5 ml filtered artificial seawater. Rub the surface of the plate with a spread bar to detach adhering amoebae.
2. Transfer the liquid medium to a sterile centrifuge tube.
3. If the cell concentration does not exceed 2 x 10^6 cells/ml adjust the suspension to that concentration. To adjust the concentration, centrifuge at 600 x g for 5 min and resuspend the pellet in the volume of fresh medium required to yield 2 x 10^6.
4. While cells are centrifuging, prepare a 15% (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 filtered artificial seawater. 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.

5. Mix the cell preparation and the DMSO in equal portions. Thus, the final concentration will be at least 10^6 cells/ml and 7.5% (v/v) DMSO. The equilibration time (the time between addition of DMSO and the start of the cooling cycle) should be no less than 15 min and no longer than 30 min.
6. Dispense in 0.5 ml aliquots into 1.0 - 2.0 ml sterile plastic screw-capped cryules (special plastic vials for cryopreservation).
7. 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.)
8. The frozen preparations are stored in either the vapor or liquid phase of a nitrogen freezer.
9. 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 to a level just above the surface of the frozen material. Do not agitate the vial.
10. Immediately after thawing, aseptically remove the contents of the ampule and distribute to the center of a fresh plate of ATCC medium 994. Distribute the material evenly over the plate using a spread bar. Seal the plate with Parafilm, place upright in a moist-chamber, and incubate at 25°C. Trophozoites should be seen within 2-3 d.

Notes

Additional information on this culture is available on the ATCC web site at www.atcc.org.

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Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Neoparamoeba pemaquidensis* (Page) Page (ATCC 30735)

References

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

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Revision

This information on this document was last updated on 2025-03-18

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