Capsaspora owczarzaki Hertel et al.

30864™

Description

Strain designation: No designation
Deposited As: *Nuclearia* sp.
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 1034: Modified PYNFH medium (Available from ATCC as ATCC cat. no. 327-X)
ATCC Medium 803: M7 medium
Instructions for complete medium: Media: ATCC Medium 1034 with serum increased to 30% (ATCC Medium 1034 is available in a freeze-dried format from ATCC; Cat# 327-X)

Alternate Media: ATCC Medium 803 with serum increased to 30%

Temperature: 25°C

- **Culture system:** Axenic

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°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 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 ATCC Medium 1034 with serum increased to 30%. Incubate the flask horizontally at 25°C with the cap screwed on tightly.

Culture maintenance: Subculture every two to three weeks to T-25 flask of fresh, complete medium in the following manner:

1. Vigorously agitate the culture flask (or scrape the flask bottom using a sterile cell scraper) and aseptically transfer 0.1-0.2 mL to a new flask of ATCC medium 1034 with serum increased to 30%.
2. Incubate the flask horizontally at 25°C with the cap screwed on tightly.

Cryopreservation:

1. Harvest cells from a culture that is at or near peak density by centrifugation at 800-900 x g for 5 min. Pool the cell pellets into a single tube.
2. Adjust the concentration of cells to 2.0 x 10^7/mL. If the concentration is too low, centrifuge at 800-900 x g for 5 minutes and resuspend the cell pellet with a volume of supernatant to yield the desired concentration.
3. Prepare a 15% (v/v) sterile DMSO solution in ATCC medium 1034 as follows: Add the required volume of DMSO to a glass screw-capped test tube and place on ice. Allow the DMSO to solidify. Add the required volume of refrigerated ATCC medium 1034. Dissolve the DMSO by inverting several times. 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 10^7 and 7.5% (v/v) DMSO. The time from the mixing of the cell preparation and cryoprotective solution to the start of the freezing process should be no less than 15 min. and no more than 60 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. Use the following cooling cycle: From room temperature cool at -10°C/min to the heat of fusion; from the heat of fusion to -40°C, cool at -1°C/min. At -40°C plunge into liquid nitrogen. The cooling cycle should be initiated no less than 15 and no more than 30 minutes after the addition of DMSO to the cell preparation.
7. The frozen preparations are stored in either the vapor or liquid phase of a nitrogen refrigerator.
8. To establish a culture from the frozen state place an ampule in a water bath set at 35°C. Immerse the vial enough to cover only the frozen material. Do not agitate the vial.
9. Immediately after thawing, do not leave in the water bath, aseptically remove the entire contents of the vial and transfer to a T-25 flask containing 10 mL ATCC Medium 1034 with serum increased to 30%.
10. Incubate the flask horizontally at 25°C with the cap screwed on tightly.

Notes

This axenic culture may exhibit particulates and/or debris which can appear to increase as the culture ages; this is normal and is attributed to some secretory activity on the part of the amoebae themselves.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Capsaspora owczarzaki* Hertel et al. (ATCC 30864)

References

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

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