Tetraselmis sp.

PRA-361™

Description

Strain designation: PB25
Deposited As: *Tetraselmis* sp.

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 2846: F/2 Medium
Instructions for complete medium: Growth with mixed bacterial flora
Temperature: 15-25°C
Atmosphere: Aerobic
Culture system: Xenic
Incubation: With mixed bacteria

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.** To assure viability, immediately loosen the test tube cap and incubate upright at 15-25°C for at least

one hour before observing the culture. There should be numerous active trophozoites in suspension. If the numbers are low the culture may have been exposed to temperature extremes in transit. Regardless of the state of the culture, aseptically transfer a 0.5 mL aliquot to a T-25 tissue culture flask containing 10 mL fresh medium. Incubate the culture at 15-25°C under a 14 hour light (~50 µEinsteins/m^2/s irradiance)/10 hour dark cycle with the cap screwed on tightly.

Culture maintenance: Subculture at peak density (approximately every 3 wks) to a fresh T-25 flask of complete medium in the following manner:

1. Vigorously agitate the flask and aseptically transfer 0.5 mL to a T-25 tissue culture flask containing 10 mL fresh medium.
2. Incubate with the cap tightly sealed at 15-25°C.

Reagents for cryopreservation: Reagents

Cryoprotective Solution

DMSO, 1.5 mL

Fresh complete growth medium, 8.5 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 the cells from a culture that is at or near peak density by centrifuging at 400 x g for 5 minutes.
3. Adjust the concentration to between 2 x 10^5 and 2 x 10^6 cells/mL with fresh medium. If the concentration is too low, centrifuge at 400 x g for 5 min and resuspend the pellet in the volume of fresh medium required to yield the desired concentration.
4. Mix the cell preparation and the DMSO in equal portions. 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 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. 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 the vial in a 35°C water bath until thawed (2-3 min). 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 containing 10 mL fresh medium.
9. Incubate the culture at 15-25°C under a 14 hour light (~50 µEinsteins/m^2/s irradiance)/10 hour dark cycle with the cap screwed on tightly.

Alternative Thawing Procedure

1. Aseptically add 0.5 mL of fresh medium to the frozen ampule. Immediately 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 remove the contents of the ampule and gently add the material to the edge of a 20 x 100 mm petri plate containing ATCC Medium 919 (non-nutrient agar) and position on a 15 degree slant. The cell suspension will pool at the edge of the plate.
3. Continue to double the volume of the cell suspension at 10 minute intervals by dropwise addition of fresh medium. When the volume reaches 16.0 mL place the plate in a horizontal position and incubate at 15-25°C under a 14 hour light (~50 µEinsteins/m^2/s irradiance)/10 hour dark cycle.
4. Once the culture has been established subculture into a T-25 flask and 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: *Tetraselmis* sp. (ATCC PRA-361)

References

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

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