Diaphanoeca grandis Ellis

50111™

Description

Strain designation: 1/19/82 NB (3)
Deposited As: *Diaphanoeca grandis* Ellis
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 1405: HESNW medium
ATCC Medium 1361: Marine flagellate medium
Instructions for complete medium:
ATCC® Medium 1405 HESNW medium (augmented with two sterile rice grains added to culture flask)
Temperature: 4-18°C
Incubation: grown with mixed bacteria

Handling Procedures

Handling of Test Tube Culture on Arrival

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 room temperature before handling.** To assure viability, immediately incubate upright at 4-18°C (~10°C is optimal) for at least one hour before observing the culture. There should be numerous trophozoites in the culture: Most are likely to be attached to the glass surface, but others may be actively swimming. If the numbers are low the culture may have been exposed to temperature extremes in transit. Regardless of the state of the culture, vigorously agitate the tube (or rub the inside surface of the tube with a sterile swab) and aseptically transfer a 0.5 ml aliquot to a T-25 tissue culture flask containing 10 ml of ice-cold ATCC medium 1405 and two sterile rice grains. Incubate the parent and daughter cultures at 4-18°C with caps screwed on tightly (keep tube upright during incubation).

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

1. Vigorously agitate the flask (or scrape the flask bottom using a sterile cell scraper) and aseptically transfer 0.5 ml from a growing culture to a T-25 tissue culture flask containing 10 ml of ice-cold ATCC medium 1405 and two sterile rice grains.
2. Incubate flask at 4-18°C with the cap on tightly.

Reagents for cryopreservation:

Cryoprotective Solution
DMSO, 2.0 ml
Fresh growth medium w/o bacteria, 8.0 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. Cool on ice prior to use.
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, ice-cold 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. **Do not allow ampule to overheat.** 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 of ice-cold ATCC medium 1405 and two sterile rice grains.
9. Incubate at 4-18°C with the cap screwed on tightly.
10. Once the culture is established, 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: *Diaphanoeca grandis* Ellis (ATCC 50111)

References

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

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Revision

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