ATCC

Product Sheet

Diaphanoeca grandis (ATCC® 50111™)

Please read this FIRST

Biosafety Level
1

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: *Diaphanoeca grandis* (ATCC® 50111™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Strain Designation: 1/19/82 NB (3)
Deposited Name: *Diaphanoeca grandis* Ellis
Depositor: PG Davis
Isolation:
Narragansett Bay, RI, 1982

Propagation

Growth Conditions
Max Temperature: 18.0°C
Min Temperature: 4.0°C
Duration: grown with mixed bacteria
Protocol: ATCCNO: 50111 SPEC: Upon receipt, remove test tube from package and immediately place it between 4 and 18C. Do not allow it to reach room temperature. Also place a T-25 tissue culture flask containing 10 ml of fresh medium and 1 or 2 autoclaved rice grains at the same temperature. Allow the vessels to equilibrate for 2-3 hours and then aseptically transfer a 0.1 ml aliquot from the test tube to the flask. Maintain both culture vessels. Transfer every 14-21 days when maintained at 10C.

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)

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.

Cryopreservation

Cryoprotective Solution

DMSO	2.0 ml
Fresh growth medium w/o bacteria	8.0 ml

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.

References

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Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

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

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