Hartaetosiga balthica (Wylezich and Karpov) Carr, Richter and Nitsche

50964™

Description

Strain designation: BSA-02190019
Deposited As: *Monosiga gracilis* Kent
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

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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 1525: Seawater 802 medium
Instructions for complete medium: ATCC Medium 1525 may be pre-inoculated with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™) for better growth
Temperature: 25°C
Atmosphere: Aerobic
Culture system: Xenic

Handling Procedures

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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 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. Add the thawed contents to a T-25 flask containing 10 mL of ATCC medium 1525 bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™).
3. Incubate with the cap tightly sealed at 20-25°C.

Culture maintenance:

Subculture at peak density (approximately every 10-14 d) 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 0.5-1.0 mL to a T-25 tissue culture flask containing 10 mL complete medium. If an organism cannot be easily suspended using agitation alone, rub the surface of the flask with a sterile cotton swab, cell scraper, or a rubber policeman before agitation.
2. Incubate with the cap tightly sealed at 20-25°C.

Reagents for cryopreservation: Cryoprotective Solution

DMSO, 2.0 mL

Fresh growth medium, 8.0 mL

Cryopreservation:

Harvest and Preservation

1. To achieve the best results, set up cultures with several different inocula (i.e., 0.5 mL, 1.0 mL, and 2.0mL). Harvest cultures and pool when the culture that received the lowest inoculum is at or near peak density. Use a sterile cotton swab, cell scraper, or a rubber policeman to detach adherent organisms
2. Adjust the concentration to approximately 1 x 10^7 cells/mL by centrifugation at 700-800 x g for 5 min and resuspend the pellet in the volume of fresh

medium required to yield the desired concentration.

3. While cells are centrifuging prepare a 20% (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 medium. 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.
4. Mix the cell preparation and the DMSO in equal portions. Thus, the final concentration will be approximately 5 x 10^6 cells/mL and 10% (v/v) DMSO. 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 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.)
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. 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 tissue culture flask containing 10 mL ATCC medium 1525 bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™).
9. Incubate with the cap tightly sealed at 20-25°C.
10. Once the culture is established, follow the protocol for maintenance of culture.

Material Citation

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If use of this material results in a scientific publication, please cite the material in the following manner: *Hartaetosiga balthica* (Wylezich and Karpov) Carr, Richter and Nitsche (ATCC 50964)

References

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

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