



Product Sheet

# *Hartaetosiga balthica* (ATCC® 50964™)

Please read this FIRST

Storage Temp.  
**Frozen Cultures:**  
**-70°C for 1 week;**  
**liquid N<sub>2</sub> vapor**  
**for long term**  
**storage**



**Freeze-dried**  
**Cultures:**  
**2-8°C**

**Live Cultures:**  
**See Protocols**  
**section for**  
**handling**  
**information**



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: *Hartaetosiga balthica* (ATCC® 50964™)

American Type Culture Collection  
PO Box 1549  
Manassas, VA 20108 USA  
[www.atcc.org](http://www.atcc.org)

800.638.6597 or 703.365.2700  
Fax: 703.365.2750  
Email: [Tech@atcc.org](mailto:Tech@atcc.org)

Or contact your local distributor

## Description

**Strain Designation:** BSA-02190019

**Deposited Name:** *Monosiga gracilis* Kent

**Depositor:** TA Nerad

**Isolation:** Salt marsh sediment, Cattleshed Marsh, eastern shore of Virginia, 2000

## Propagation

### Growth Conditions

**Temperature:** 25°C

**Atmosphere:** Aerobic

**Culture System:** Xenic

### 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.

## Protocols

### 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.

## Cryopreservation

### Reagents

#### Cryoprotective Solution

DMSO, 2.0 mL

Fresh growth medium, 8.0 mL

### 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 2.0 mL). 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  $1 \times 10^7$  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



## Product Sheet

# *Hartaetosiga balthica* (ATCC® 50964™)

### Please read this FIRST

Storage Temp.  
**Frozen Cultures:**  
**-70°C for 1 week;**  
**liquid N<sub>2</sub> vapor**  
**for long term**  
**storage**



**Freeze-dried**  
**Cultures:**  
**2-8°C**

**Live Cultures:**  
**See Protocols**  
**section for**  
**handling**  
**information**



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: *Hartaetosiga balthica* (ATCC® 50964™)

American Type Culture Collection  
PO Box 1549  
Manassas, VA 20108 USA  
[www.atcc.org](http://www.atcc.org)

800.638.6597 or 703.365.2700  
Fax: 703.365.2750  
Email: [Tech@atcc.org](mailto:Tech@atcc.org)

Or contact your local distributor

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.

- Mix the cell preparation and the DMSO in equal portions. Thus, the final concentration will be approximately 5 x 10<sup>6</sup> 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.
- Dispense in 0.5 mL aliquots into 1.0 - 2.0 mL sterile plastic screw-capped cryules (special plastic vials for cryopreservation).
- 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.)
- The frozen preparations are stored in either the vapor or liquid phase of a nitrogen freezer.
- 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™).
- Incubate with the cap tightly sealed at 20-25°C.
- Once the culture is established, follow the protocol for maintenance of culture.



### References

References and other information relating to this product are available online at [www.atcc.org](http://www.atcc.org).



### 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.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

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](http://www.atcc.org)

Additional information on this culture is available on the ATCC web site at [www.atcc.org](http://www.atcc.org).

© ATCC 2017. All rights reserved. ATCC is a registered trademark of the American Type Culture Collection. [10/13]