



# ***Helgoeca nana* Leadbeater**

**50073<sup>TM</sup>**

## Description

**Strain designation:** 3/25/81 NB(2)

**Deposited As:** *Acanthoecopsis unguiculata* Thomsen

**Type strain:** No

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## Storage Conditions

**Product format:** Frozen

**Storage conditions:** -80°C or colder for 1 week, vapor phase of liquid nitrogen for long-term storage

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## 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.

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## 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.



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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.

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### **Certificate of Analysis**

For batch-specific test results, refer to the applicable certificate of analysis that can be found at [www.atcc.org](http://www.atcc.org).

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### **Growth Conditions**

#### **Medium:**

ATCC Medium 1405: HESNW medium

ATCC Medium 1361: Marine flagellate medium

#### **Instructions for complete medium:**

**Media:** ATCC® medium 2417 Marine Choanoflagellate medium (augmented with two sterile rice grains added to culture flask) inoculated with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831)

**Alternate media:** ATCC® medium 1405 HESNW medium, ATCC Medium 1361 Marine Flagellate medium (both augmented with two sterile rice grains added to culture flask and inoculated with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831))

**Temperature:** 4°C

**Culture system:** Xenic

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**Incubation:** Grown with mixed bacteria

## **Handling Procedures**

### **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 ampules 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.

Note: Care must be taken to maintain this organism at 4°C at all times. All media used should be pre-chilled to 4°C. The culture may be briefly examined under the microscope at room temperature but every effort should be made to keep the organism at 4°C (e.g. transport culture on ice to and from the incubator)

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. Care must be taken 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 of chilled ATCC medium 2417 (augmented with two sterile rice grains added to culture flask) bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831).
3. Immediately incubate flask at 4°C with the cap tightly sealed.

### **Culture maintenance:**

Note: Care must be taken to maintain this organism at 4°C at all times.

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

1. Vigorously agitate the flask and aseptically transfer 0.5 ml from a growing culture to a T-25 tissue culture flask containing 10.0 ml of ATCC medium 2417 (augmented with two sterile rice grains added to culture flask) bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831)
2. Incubate flask at 4°C with the cap on tightly.

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DMSO, 1.5 ml

Fresh growth medium w/o bacteria, 8.5 ml

Note: Care must be taken to maintain this organism at 4°C at all times. Media and cell suspension should be kept on ice during entire process.

**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. Chill to 4°C.
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  $2 \times 10^6$ /ml in fresh 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 leave in water bath after thawing. Aseptically remove the contents of the ampule and inoculate into a T-25 tissue culture flask containing 10 ml chilled ATCC medium 2417 bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831).
9. Incubate at 4°C with the cap screwed on tightly.
10. Once the culture is established, vigorously agitate the flask and aseptically transfer 0.5 ml to 10.0 ml of bacterized ATCC medium 2417.
11. Follow the protocol for maintenance of culture.

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### **Material Citation**

If use of this material results in a scientific publication, please cite the material in the following manner: *Helgoeca nana* Leadbeater (ATCC 50073)

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### **References**

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

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