



Product Sheet

# *Acanthoeca spectabilis* (ATCC® PRA-103™)

## 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: *Acanthoeca spectabilis* (ATCC® PRA-103™)

## Description

**Strain Designation:** Ellis

**Depositor:** B Leadbeater

**Isolation:**

April 18, 1996 from salt marsh in Swan Bay, Queenscliffe, Australia.

## Propagation

### Growth Conditions

**Temperature:** 20.0°C

**Growth condition:** monoxenic with *Enterobacter aerogenes* ATCC 13048.

**Protocol:** Thaw the frozen material in a 35.0°C water bath and transfer the contents into a T25 flask containing 10 ml of growth medium. Flagellates should be observed within 4-6 days. Subculture on a weekly basis by scraping the bottom of the flask with a cell scraper and transferring one tenth of the culture into a new flask with medium.

### Medium

ATCC® Medium 1525: Seawater 802 medium

### Instructions for Complete Medium

ATCC® Media 1525 and 1405, combined in equal parts and inoculated with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831) or *Enterobacter aerogenes* (ATCC® 13048)

### Culture Maintenance

Subculture every two weeks to a fresh T-25 flask of bacterized 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 10.0 ml of an equal-parts mixture of ATCC media 1525 and 1405 bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831) or *Enterobacter aerogenes* (ATCC® 13048).
2. Incubate flask at 20-25°C with the cap on tightly.

## Cryopreservation

### Cryoprotective Solution

DMSO	1.5 ml
Fresh growth medium w/o bacteria	8.5 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.
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. 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 an equal-parts mixture of ATCC media 1525 and 1405 bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831) or *Enterobacter aerogenes* (ATCC® 13048).
9. Incubate at 20-25°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 fresh bacterized medium.
11. Follow the protocol for maintenance of culture.

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## 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.

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## Disclaimers

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

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

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