



Acanthoeca spectabilis

PRA-103™

Description

Strain designation: Ellis

Type strain: No

Storage Conditions

Product format: Frozen

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 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

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

Temperature: 20°C

Culture system: Xenic

Incubation: With *Enterobacter aerogenes* ATCC 13048 as a food source.

Handling Procedures

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

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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.

Reagents for cryopreservation:RM-9 Media for cryopreservation of *Tetrahymena*

Proteose Peptone (Difco 0120)	5.0 g
Tryptone	5.0 g
K ₂ HPO ₄	0.2 g
Glucose	1.0 g
Liver extract	0.1 g
Glass distilled water	1.0 L

Dissolve components in glass distilled H₂O and autoclave.

Dryl's Salt Solution

0.1 M NaH ₂ PO ₄ · 3H ₂ O	10.0 ml
0.1 M Na ₂ HPO ₄ · 7H ₂ O	10.0 ml
0.1 M Sodium citrate · 2H ₂ O	15.0 ml
0.1 M CaCl ₂ · 2H ₂ O	15.0 ml
Distilled water	950.0 ml

Add the first 3 components to the distilled H₂O and mix thoroughly.

Add the CaCl₂ solution and mix thoroughly.

(Adding the solutions in the order indicated will avoid the precipitation of Ca salts.)

Cryopreservation: 1. Transfer *tetrahymena* from usual growth medium to RM-9 medium and allow to grow to near peak density.

2. Harvest cells from a culture that is at or near peak density by filtration and

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centrifugation at 800 x g for 5 min.

3. Adjust the concentration of cells at least 2×10^6 /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 $-1^\circ\text{C}/\text{min}$ to -40°C . If freezing unit can compensate for the heat of fusion, maintain rate at $-1^\circ\text{C}/\text{min}$ 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 $-1^\circ\text{C}/\text{min}$.)
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 $20\text{-}25^\circ\text{C}$ 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.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Acanthoeca spectabilis* (ATCC PRA-103)

References

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

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