Parauronema acutum Buddenbrock

50307™

Description

Strain designation: 110-3 killer
Deposited As: *Parauronema acutum* Buddenbrock
Type strain: No

Storage Conditions

Product format: Test tube
Storage conditions: See handling procedure

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 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 1651: MA medium
Temperature: 25°C
Culture system: Axenic

Handling Procedures

Handling of Live Culture

This strain is routinely shipped as a growing culture in a glass 16 x 125 mm screw-capped test tube. The volume of the cell suspension is approximately 5 mL. When the culture arrives remove it promptly from the shipping container. **Do not store the culture at refrigeration temperatures before handling.** To assure viability,

immediately loosen the test tube cap and incubate upright at 25°C for at least one hour before observing the culture. There should be numerous active trophozoites in suspension. If the numbers are low the culture may have been exposed to temperature extremes in transit. Regardless of the state of the culture, aseptically transfer a 0.5 mL aliquot to a 16 x 125 mm screw-capped test tube containing 5 mL of sterile ATCC medium 1651. Incubate the parent and daughter cultures upright with the caps on loosely at 25°C.

Culture maintenance:

1. Screw the cap on tightly and vigorously agitate the culture.
2. Aseptically transfer a 0.1-0.25 mL aliquot to 5 mL of fresh medium in a 16 x 125 mm screw-capped test tube or T-25 tissue culture flask.
3. Incubate at 25°C (test tubes are incubated upright with the cap loosened one-half turn).
4. Subculture every 3-5 days.

Cryopreservation:

1. Harvest cells from a culture that is at or near peak density by centrifugation at 800 x g for 5 min.
2. Adjust the concentration of cells to 2 x 10^6 /mL in fresh medium.
3. While cells are centrifuging prepare a 22% (v/v) solution of sterile DMSO in fresh medium.
4. Mix the cell preparation and the 22% DMSO in equal portions. Thus, the final concentration will be 10^6 cells/mL and 11% (v/v) DMSO. The time from the mixing of the cell preparation and DMSO stock solution to the beginning of the freezing process should be no less than 15 min and no greater than 60 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 should be stored in either the vapor or liquid phase of a nitrogen refrigerator. Frozen preparations stored below -130°C are stabile indefinitely. Those stored at temperatures above -130°C are progressively less

stabile as the storage temperature is elevated. Vials should not be stored above -55°C.

8. 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 thawed.
9. Immediately after thawing, do not leave in the water bath, gently remove the contents of the ampule with a Pasteur pipette and expel slowly into a 16 x 125 mm screw-capped test tube or T-25 tissue culture flask. Incubate at room temperature (approx. 25°C) for 15 min.
10. At 15 min intervals add 0.25 mL of ATCC medium 1651 dropwise. Continue until the final volume is 2.0 mL.
11. Allow the culture to remain undisturbed for 15 min.
12. Add 0.5 mL of medium dropwise at 15 min intervals until the volume is 4.0 mL.
13. Allow the culture to remain undisturbed overnight.
14. On the morning of day 2 slowly add 4.0 mL of ATCC medium 1651. Allow the culture to remain undisturbed overnight.
15. When the culture is established, 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: *Parauronema acutum* Buddenbrock (ATCC 50307)

References

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

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Revision

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