Tokophrya lemnarum (Stein) Entz

50033™

Description

Strain designation: Oneonta
Deposited As: *Tokophrya lemnarum* (Stein) Entz
Type strain: No

Storage Conditions

Product format: Test tube

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 1323: Page's balanced salt solution (PBS)
Instructions for complete medium: ATCC Medium 1323
Temperature: 25°C
Incubation: ATCCNO: 50032 SPEC: Food source, Paramecium tetraurelia ATCC 30567, not supplied.

Handling Procedures

Culture maintenance:
Periodically add prey organisms as follows:

1. Maintain growing cultures of *Paramecium* separately at 25°C in T-25 tissue culture flasks containing 10 ml ATCC medium 802 bacterized with *Klebsiella pneumoniae*

subsp. *pneumoniae* (ATCC® 700831) or *Enterobacter aerogenes* (ATCC® 13048).

2. Prepare washed *Paramecium* as follows: Remove 5-10 ml from a culture at or near peak density, centrifuge at 300 x g for 5 min, quickly remove most of the supernatant (leaving approx. 1 ml), then resuspend cells in 10 ml ATCC medium 1323. Centrifuge and resuspend cells again as above. Repeat this washing step at least twice.

3. When the *Tokophrya* have consumed all prey *Paramecium*, add 0.5-2 ml of washed *Paramecium* prepared in step 2. The feeding interval will depend on the number of suctorians present and the culture density of the washed prey.

4. The *Tokophrya* may be passaged to a new petri plate or T-25 flask by gently rubbing the agar surface with a spread bar to dislodge attached suctorians, then transferring 0.5 to 2 ml to a fresh petri plate or T-25 flask containing a bed of non-nutrient agar (ATCC medium 919) and 10 ml ATCC medium 1323. Incubate the culture at 20-25°C, feeding periodically with washed *Paramecium*.

Reagents for cryopreservation:
Cryoprotective Solution

DMSO	2.0 ml
Fresh growth medium w/o bacteria	8.0 ml

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.

2. Harvest *Tokophrya* cells from a culture that has recently passed peak density by centrifugation at 250-300 x g for 5 min.

3. Adjust the concentration of cells to at least 2 x 10^4/ml in fresh medium.

4. Mix the cell preparation and the cryoprotective solution in equal portions by adding the cryoprotective solution to the cell suspension in 3 equal aliquots at 2 min. intervals.

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 transfer to a petri plate or T-25 tissue culture flask containing a bed of non-nutrient agar (ATCC medium 919) and 10 ml ATCC medium 1323.

9. Aseptically transfer 0.5-2.0 ml of washed *Paramecium* to the petri plate or T-25 flask (see section on MAINTENANCE OF CULTURE). Incubate the culture at 20-25°C.

Once the culture is established, follow the protocol for maintenance of culture.

Notes

This strain must be fed with live *Paramecium* (i.e., ATCC® 30567 or similar, not provided). The *Paramecium* should be maintained separately and fed to *Tokophrya* at regular intervals. Overfeeding of *Tokophrya* may result in monster formation. Attempt to maintain a ratio of 2-3 prey organisms per each suctorian. If the number of abnormal suctorians is high, reduce the feeding interval or passage the culture.

This strain of *Tokophrya lemnarum* is mating type I. The culture is polyxenic and contains mixed bacterial flora.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Tokophrya lemnarum* (Stein) Entz (ATCC 50033)

References

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

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Revision

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