Sorogena stoianovitchae Bradbury and Olive

50031™

Description

Strain designation: PNG 76-73
Deposited As: *Sorogena stoianovitchae* Bradbury and Olive
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 1330: Sorogena medium
Temperature: 20-25°C

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 5-6 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 a number of attached *Sorogena*

sorocarps near the fluid meniscus. If the numbers are low the culture may have been exposed to temperature extremes in transit. Regardless of the state of the culture, suspend attached sorocarps by placing the tube on ice for 10 min, then rub the inside surface of the tube with a sterile inoculating loop or cotton swab to detach cells. Aseptically transfer the entire contents of the tube to a petri plate or T-25 tissue culture flask containing a bed of agar medium with 6-8 mL additional liquid overlay (ATCC medium 1330). Incubate the culture at 20-25°C under a 14 hour light (~50 µEinsteins/m^2/s irradiance)/10 hour dark cycle.

Culture maintenance:

1. When the *Sorogena* have sufficiently reduced the number of prey *Colpoda* in the culture, their ability to aggregate and form sorocarps is diminished, and both predator and prey form cysts. To revive an encysted culture, the liquid overlay should be aseptically removed and replaced with fresh medium, which will stimulate new bacterial growth and encourage excystment of *Colpoda* and then *Sorogena*.
2. Incubate the culture at 20-25°C under a 14 hour light (~50 µEinsteins/m^2/s irradiance)/10 hour dark cycle. Active trophozoites (ciliates) of both *Colpoda* and *Sorogena* should be observed within 2-3 days. *Sorogena* sorocarps (cysts) should form just above the air-liquid interface in 5-7 days.
3. To encourage faster proliferation of *Sorogena*, additional *Colpoda* sp. may be added to the culture from a separate culture of the prey organism kept in parallel (i.e., ATCC® 30920™ or similar, not provided).
4. The *Sorogena* 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 sorocarps and/or cysts, then transferring 0.5 to 2 mL to a fresh petri plate or T-25 flask containing a bed of agar medium and 10-15 mL total liquid overlay (ATCC medium 1330).
5. Incubate the culture at 20-25°C under a 14 hour light (~50 µEinsteins/m^2/s irradiance)/10 hour dark cycle. Active trophozoites (ciliates) of both *Colpoda* and *Sorogena* should be observed within 2-3 days. *Sorogena* sorocarps (cysts) should form just above the air-liquid interface in 5-7 days. Several cycles of growth and encystment may be required in order for the *Sorogena* culture to produce fruiting bodies.

Reagents for cryopreservation: Cryoprotective Solution

DMSO, 1.5 mL

Fresh growth medium w/o bacteria, 8.5 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 *Sorogena* and *Colpoda* cysts from a culture that has recently passed peak density by centrifugation at 1000 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 agar medium and 10-15 mL total liquid overlay (ATCC medium 1330).
9. Optionally, aseptically transfer 0.2-0.5 mL from a growing culture of *Colpoda* sp. to the petri plate or T-25 flask (See section on Culture Maintenance).
10. Incubate the culture at 20-25°C under a 14 hour light (~50 µEinsteins/m^2/s irradiance)/10 hour dark cycle. Active trophozoites (ciliates) of both *Colpoda* and *Sorogena* should be observed within 2-3 days. Once the culture is established, follow the protocol for maintenance of culture.

Notes

This strain feeds on *Colpoda* sp. (both organisms are included in the culture). This is a xenic culture that contains bacteria.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Sorogena stoianovitchae* Bradbury and Olive (ATCC 50031)

References

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

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