Actinophrys sol Ehrenberg

50937™

Description

Deposited As: *Actinophrys sol* Ehrenberg
Type strain: No

Storage Conditions

Product format: Frozen
Storage conditions: -80°C or colder for 1 week, vapor phase of liquid nitrogen for long-term storage

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 2454: Actinophrys medium
Temperature: 20-25°C

Handling Procedures

Storage and Culture Initiation

Frozen ampules packed in dry ice should either be thawed immediately or stored in liquid nitrogen. If liquid nitrogen storage facilities are not available, frozen ampoules may be stored at or below -70°C for approximately one week. **Do not under any circumstance store frozen ampules at refrigerator freezer temperatures (generally -20°C).** Storage of frozen material at this temperature will result in the death of the

culture.

1. 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 it is thawed. Do not allow ampule to overheat.
2. Add the thawed contents to a petri plate or T-25 tissue culture flask optionally containing a bed of non-nutrient agar (ATCC medium 919) and 10 mL ATCC medium 2454.
3. Aseptically transfer an appropriate volume of washed *Chlorogonium* to the petri plate or T-25 flask (see section on MAINTENANCE OF CULTURE below). Incubate the culture at 20-25°C

Culture maintenance:

Periodically add prey organisms as follows:

1. Maintain growing cultures of *Chlorogonium* separately at 25°C in T-25 tissue culture flasks containing 10 mL appropriate fresh medium.
2. Prepare washed *Chlorogonium* as follows: Remove 5-10 mL from a culture at or near peak density, centrifuge at 500-600 x g for 5 min, quickly remove most of the supernatant (leaving approx. 1 mL), then resuspend cells in 10 mL ATCC medium 2454. Centrifuge and resuspend cells again as above. This washing step should be performed at least twice.
3. Add an appropriate volume of washed *Chlorogonium* prepared in step 2 when the number of *Actinophrys* begins to decline and/or significant cyst formation occurs. Add approximately 100-150 prey organisms per heliozoan for the initial inoculation of prey into a new *Actinophrys* culture. Attempt thereafter to maintain a *Chlorogonium* cell density of approximately 2-3 x 10^4/mL for optimal growth of *Actinophrys*. The feeding interval will depend on the number of heliozoans present and the culture density of the washed prey.
4. The *Actinophrys* may be passaged to a new petri plate or T-25 flask by gently rubbing the agar or plastic surface with a spread bar to dislodge attached heliozoans, then transferring 0.5 to 2 mL to a fresh petri plate or T-25 flask optionally containing a bed of non-nutrient agar (ATCC medium 919) and 10 mL ATCC medium 2454. Incubate the culture at 20-25°C, feeding periodically with washed *Chlorogonium*.

Reagents for cryopreservation: Cryoprotective Solution
DMSO 1.0 mL

Betaine 1.0 mL
Fresh growth medium 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 *Actinophrys* cells from a culture that has recently passed peak density by centrifugation at 300-400 x g for 5 min.
3. Adjust the concentration of cells to 1-2 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 2 equal aliquots approximately 1 min apart.
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 optionally containing a bed of non-nutrient agar (ATCC medium 919) and 10 mL ATCC medium 2454
9. Aseptically transfer an appropriate volume of washed Chlorogonium 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 *Chlorogonium elongatum* (*i.e.*, ATCC® 50936™ or

similar, not provided). The *Chlorogonium* should be maintained separately and fed to *Actinophrys* at regular intervals.

This culture is monoxenic, free from bacterial flora.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Actinophrys sol* Ehrenberg (ATCC 50937)

References

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

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