Amoeboaphelidium protococcarum Gromov and Mamkaeva

50289™

Description

Strain designation: X-1
Deposited As: *Amoeboaphelidium protococcarum* Gromov and Mamkaeva
Type strain: Yes

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.

Amoeboaphelidium protococcarum Gromov and Mamkaeva

50289

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 5: Sporulation agar
Temperature: 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 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 on a 15° horizontal slant at 25°C for at least one hour before observing the culture. There should be numerous active

trophozoites attached to the tube or floating 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, suspend trophozoites by rubbing the inside surface of the tube with a sterile cotton swab and aseptically transfer a 0.5 mL aliquot to the surface of a 20 x 100 mm Petri plate containing a growing culture of *Scenedesmus obliquus* (i.e., ATCC® 11457™ or similar) on 20 mL of ATCC medium 5 agar. Wrap the plate culture with parafilm and incubate upright under a 14 hour light (~50 µEinsteins/m^2/s irradiance)/10 hour dark cycle. Alternatively, transfer the 0.5 mL aliquot to a 16 x 125 mm screw-capped test tube containing a growing culture of *Scenedesmus obliquus* in 5 mL of ATCC Medium 5 broth. Incubate the tube on a 15º horizontal slant with the cap screwed on loosely (loosened one half turn) at 25°C under the same light/dark cycle as specified for a plate culture.

Note: In order for the *Amoeboaphelidium* to more successfully parasitize the host alga, it may be helpful to use agar media.

Culture maintenance:

1. For a plate culture, transfer cells with an inoculating loop to a plate of fresh agar medium from a growing culture at or near peak density. For a broth culture, inoculate a tube of fresh broth medium with 0.3 to 0.5 mL from a growing culture at or near peak density.
2. Incubate at 25°C under a 14 hour light (~50 µEinsteins/m^2/s irradiance)/10 hour dark cycle. In the case of a broth culture, screw tube cap on loosely (loosened one-half turn) and incubate on a 15° horizontal slant.
3. Subculture as necessary (typically every 1-2 wks).

Reagents for cryopreservation: ATCC Medium 1323 (Page's Balanced Saline)
Solution 1 (see below) 500.0 mL
Solution 2 (see below) 500.0 mL

Solution 1
Na_2HPO_4, 0.142 g
KH_2PO_4, 0.136 g
Distilled H_2O, 500.0 mL

Solution 2
$MgSO_4 \cdot 7H_2O$, 4.0 mg
$CaCl_2 \cdot 2H_2O$, 4.0 mg
NaCl, 0.120 g

Amoeboaphelidium protococcarum Gromov and Mamkaeva

50289

Distilled H_2O, 500.0 mL

Autoclave solutions 1 and 2 separately at 121°C. Combine the two solutions when cooled to room temperature.

Cryopreservation:

1. Allow amoebae to encyst. Harvest cysts from a culture that has recently passed peak density by centrifugation at 800 x g for 5 min.
2. Adjust the concentration of cysts to 2 x 10^6 - 2 x 10^7/mL in fresh medium.
3. While cysts are centrifuging prepare a 20% (v/v) solution of sterile DMSO in fresh ATCC medium 1323 (Page's Balanced Saline).
4. Mix the cell preparation and the 20% DMSO solution in equal portions. Thus, the final concentration will be 10^6 - 10^7 cells/mL and 10% (v/v) DMSO. The time from the mixing of the cell preparation and DMSO cryoprotective 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 establish a culture from the frozen state place an ampule in a water bath set at 35°C. Immerse the vial to a level just above the surface of the frozen material. Do not agitate the vial.
9. Immediately after thawing, do not leave in the water bath, aseptically remove the contents of the ampule and add to the surface of an ATCC medium 5 agar plate containing a growing culture of *Scenedesmus obliquus*. Alternatively, transfer the thawed contents to a 16 x 125 mm screw-capped test tube containing a growing culture of *Scenedesmus obliquus* in 5 mL of ATCC medium

5 broth.

10. Incubate the culture at 50-100 µEinsteins/m^2/s irradiance at 25°C. Maintain under a 14/10h light-dark photoperiod.

Notes

This strain is an endoparasite and must be fed with live *Scenedesmus obliquus* (i.e., ATCC® 11457™ or similar, not provided). The *Scenedesmus* should be maintained separately and fed to *Amoeboaphelidium* at regular intervals. The feeding interval will depend on the number of amoebae present and the culture density of the host alga. If the number of amoebae is high, increase the feeding interval or passage the culture. *Amoeboaphelidium* will form cysts once the host alga population has been sufficiently depleted.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Amoeboaphelidium protococcarum* Gromov and Mamkaeva (ATCC 50289)

References

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

Warranty

The product is provided 'AS IS' and the viability of ATCC® products is warranted for 30 days from the date of shipment, provided that the customer has stored and handled the product according to the information included on the product information sheet,

website, and Certificate of Analysis. For living cultures, ATCC lists the media formulation and reagents that have been found to be effective for the product. While other unspecified media and reagents may also produce satisfactory results, a change in the ATCC and/or depositor-recommended protocols may affect the recovery, growth, and/or function of the product. If an alternative medium formulation or reagent is used, the ATCC warranty for viability is no longer valid. Except as expressly set forth herein, no other warranties of any kind are provided, express or implied, including, but not limited to, any implied warranties of merchantability, fitness for a particular purpose, manufacture according to cGMP standards, typicality, safety, accuracy, and/or noninfringement.

Disclaimers

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. Any proposed commercial use is prohibited without a license from ATCC.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate or complete and the customer bears the sole responsibility of confirming the accuracy and completeness of any such information.

This product is sent on the condition that the customer is responsible for and assumes all risk and responsibility in connection with the receipt, handling, storage, disposal, and use of the ATCC product including without limitation taking all appropriate safety and handling precautions to minimize health or environmental risk. As a condition of receiving the material, the customer agrees that any activity undertaken with the ATCC product and any progeny or modifications will be conducted in compliance with all applicable laws, regulations, and guidelines. This product is provided 'AS IS' with no representations or warranties whatsoever except as expressly set forth herein and in no event shall ATCC, its parents, subsidiaries, directors, officers, agents, employees, assigns, successors, and affiliates be liable for

indirect, special, incidental, or consequential damages of any kind in connection with or arising out of the customer's use of the product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of such materials.

Please see the material transfer agreement (MTA) for further details regarding the use of this product. The MTA is available at www.atcc.org.

Copyright and Trademark Information

Revision

This information on this document was last updated on 2025-08-21

Contact Information

ATCC
10801 University Boulevard
Manassas, VA 20110-2209
USA
US telephone: 800-638-6597
Worldwide telephone: +1-703-365-2700
Email: tech@atcc.org or contact your local distributor