ATCC

Product Sheet

Amoeboaphelidium protococcarum (ATCC® 50289™)

Please read this FIRST

Storage Temp.
Frozen Cultures:
-70°C for 1 week; liquid N_2 vapor for long term storage

Freeze-dried Cultures:
2-8°C

Live Cultures:
See Protocols section for handling information

Biosafety Level
1

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: *Amoeboaphelidium protococcarum* (ATCC® 50289™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Strain Designation: X-1
Deposited Name: *Amoeboaphelidium protococcarum* Gromov and Mamkaeva
Depositor: BV Gromov
Isolation: N/A

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.

Propagation

Growth Conditions
Temperature: 25°C

Medium
ATCC® Medium 5: Sporulation agar

Protocols

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 15^o 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).

Cryopreservation

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

Solution 1

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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
Distilled H_2O, 500.0 mL

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

Harvest and Preservation

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.

References

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

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

Additional information on this culture is available on the ATCC web site at www.atcc.org.