ATCC

Product Sheet

Acanthamoeba hatchetti (ATCC® 50673™)

Please read this FIRST

Biosafety Level
2

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: *Acanthamoeba hatchetti* (ATCC® 50673™)

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
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Or contact your local distributor

Description

Strain Designation: FDA-97B-MX
Deposited Name: *Acanthamoeba hatchetti* Sawyer et al.
Depositor: TK Sawyer
Isolation:
sewage dump site, Philadelphia, 1978

Propagation

Growth Conditions
Temperature: 25.0°C
Protocol: ATCCNO: 50655 SPEC: The strain is distributed as a frozen stabilate. See general instructions for thawing and storage of frozen material before proceeding. Remove the frozen ampule from the dry ice and transfer directly to a 35C water bath. After thawing the ampule, transfer the contents to the surface of an ATCC medium 997 or 711 agar plate (20 x 100 mm). Spread the material evenly over the surface of the plate with a sterile spread bar. The food bacterium, Enterobacter aerogenes ATCC 13048, is present in the thawed material. Wrap the plate with time tape or parafilm and incubate upright at 25C. Many trophozoites should be visible within 2-3 days. Transfer the culture every 28 days as follows: Aseptically remove a small block of agar (approximately 5 mm square) containing cysts and, at the edge of an ATCC medium 997 or 711 agar plate containing a lawn of Enterobacter aerogenes ATCC 13048, invert the block onto the surface. Wrap the new plate and incubate as above. The trophozoites will migrate away from the agar block to the opposite edge of the plate. Continue to subculture as above.

Medium
ATCC® Medium 711: PYB
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ATCC® Medium 997: Fresh water ameba medium

Instructions for Complete Medium
ATCC Medium 711

Culture Maintenance
1. Streak an ATCC medium 711 plate with *Enterobacter aerogenes* (ATCC® 13048) and incubate at 35°C overnight.
2. Remove an agar block (~5 mm^2), with trophozoites or cysts, from the edge of an agar plate culture and invert the block at the edge of the freshly bacterized plate.
3. Wrap the entire edge of the plate with parafilm and incubate upright at 25°C.
4. Repeat steps 1-3 at 10-14 d intervals.

Note: a monoxenic amoeba culture can be established in this manner using any suitable bacterial food source.

Cryopreservation

1. Allow the cells to encyst. To detach cysts from the plate flush the surface with 5 ml fresh ATCC medium 1323 (Page's Balanced Salt Solution). Rub the surface of the plate with a spread bar to detach adhering cysts.
2. Transfer the liquid medium to a sterile centrifuge tube.
3. If the cyst concentration does not exceed 2 x 10^6 cysts/ml adjust the suspension to that concentration. To adjust the concentration, centrifuge at 600 x g for 5 min and resuspend the pellet in the volume of fresh medium required to yield 2 x 10^6.
4. While cells are centrifuging prepare a 15% (v/v) solution of sterile DMSO as follows: Add the required volume of DMSO to a glass screw-capped test tube and place it in an ice bath. Allow the DMSO to solidify. Add the required volume of refrigerated medium. Dissolve the DMSO by inverting the tube several times. *NOTE: If the DMSO solution is not prepared on ice, an exothermic reaction will occur that may precipitate certain components of the medium.
5. Mix the cell preparation and the DMSO in equal portions. Thus, the final concentration will be at least 10^6 cysts/ml and 7.5% (v/v) DMSO. The equilibration time (the time between addition of DMSO and the start of the cooling cycle) should be no less than 15 min and no longer than 30 min.
6. Dispense in 0.5 ml aliquots into 1.0 - 2.0 ml sterile plastic screw-capped cryules (special plastic vials for

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cryopreservation).

7. 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.)
8. The frozen preparations are stored in either the vapor or liquid phase of a nitrogen freezer.
9. To establish a culture from the frozen state place an ampule in a water bath set at 35°C (2-3 min). Immerse the vial to a level just above the surface of the frozen material. Do not agitate the vial.
10. Immediately after thawing, aseptically remove the contents of the ampule and distribute to the center of a fresh plate of ATCC medium 711. Distribute the material evenly over the plate using a spread bar. Incubate at 25°C.

References

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

Biosafety Level: 2

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

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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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.

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