ATCC

Product Sheet

Acanthamoeba griffini (ATCC® 30731™)

Please read this FIRST

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: *Acanthamoeba griffini* (ATCC® 30731™)

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: S-7
Deposited Name: *Acanthamoeba griffini* Sawyer
Depositor: TK Sawyer
Isolation:
shallow-water, beach-bottom sample, New London, CT, 1962

Propagation

Growth Conditions
Temperature: 25.0°C
Duration: grown with bacteria
Protocol: ATCCNO: 30011 SPEC: This strain is distributed as a dried preparation. See the general procedures for opening a dried vial. Aseptically add 1 ml of sterile distilled water to the inner shell vial, remove the filter paper aseptically with a pair of forceps, and place it in the center of an agar plate of ATCC medium 997. Add the liquid remaining in the vial to the plate and spread it evenly over the surface of the plate. Incubate the plate at 25C. Trophozoites (amebae) should be evident within 2-3 days.

Medium
ATCC® Medium 997: Fresh water ameba medium

Instructions for Complete Medium
ATCC medium 997 inoculated with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831) or *Enterobacter aerogenes* (ATCC® 13048).

Culture Maintenance
1. Streak an ATCC medium 997 plate with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831) or *Enterobacter aerogenes* (ATCC® 13048) and incubate at 35°C overnight.
2. Remove an agar block (~5 mm^2) with trophozoites 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

Cryoprotective Solution

DMSO	1.5 ml
Dryl's solution (or similar)	8.5 ml

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 cells from a culture which is at or near peak density by adding 5 ml ATCC medium 5080 (Dryl's solution) and washing cells into suspension. Rub the surface of the plate with a spread bar to detach adhering trophozoites.
3. Adjust the concentration of cells to at least 2 x 10^6/ml in fresh medium.
4. Mix the cell preparation and the cryoprotective solution in equal portions.
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, aseptically transfer the contents of the ampule to the center of a fresh plate of ATCC medium 997. Distribute the material evenly over the plate using a spread bar.
9. Wrap the entire edge of the plate with parafilm and incubate upright at 25°C.

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Follow the protocol for maintenance of culture.

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

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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