ATCC

Product Sheet

Naegleria fowleri (ATCC® 30474™)

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
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: *Naegleria fowleri* (ATCC® 30474™)

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: PA-105
Deposited Name: *Naegleria fowleri* Carter
Depositor: JA Jamieson
Isolation: Domestic water supply, Port Augusta, South Australia, 1972

Propagation

Growth Conditions
Temperature: 30°C to 35°C
Culture System: Axenic

Medium
ATCC® Medium 1034: Modified PYNFH medium (Available from ATCC as ATCC cat. no. 327-X)
ATCC® Medium 710: Nelson's Culture Medium For Naegleria
ATCC® Medium 803: M7 medium
ATCC® Medium 902: Schuster's axenic Naegleria medium

Instructions for Complete Medium
Media: ATCC medium 1034 (ATCC medium 1034 is available in a freeze-dried format as cat. no. 327-X).
Alternate media: ATCC medium 710, ATCC medium 803, ATCC medium 902

Protocols

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 until thawed (2-3 min). Immerse the ampule just sufficiently to cover only the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically transfer contents to either a plastic screw-capped tube containing 5 mL ATCC medium 1034, or to a T-25 tissue culture flask containing 10 mL ATCC medium 1034. Incubate at 35°C with the cap screwed on tightly (incubate a test tube on a 15° horizontal slant).

Culture Maintenance

1. Vigorously agitate a culture at or near peak density and aseptically transfer 0.1-0.2 mL to a fresh tube or flask of ATCC medium 1034.
2. Incubate at 35°C with the cap screwed on tightly (incubate a test tube on a 15° horizontal slant).

Cryopreservation

Harvest and Preservation

1. Harvest cells from a culture that is at or near peak density by centrifugation at 600 x g for 5 min. Pool the cell pellets into a single tube.
2. Adjust the concentration of cells to 2.0 x 10^6/mL. If the concentration is too low, centrifuge at 600 x g for 5 minutes and resuspend the cell pellet with a volume of supernatant to yield the desired concentration.
3. Prepare a 15% (v/v) sterile DMSO solution in ATCC medium 1034 as follows: Add the required volume of DMSO to a glass screw-capped test tube and place on ice. Allow the DMSO to solidify. Add the required volume of refrigerated ATCC medium 1034. Dissolve the DMSO by inverting several times. If the DMSO solution is not prepared on ice, an exothermic reaction will occur that may precipitate certain components of the medium.
4. Mix the cell preparation and the DMSO in equal portions. Thus, the final concentration will be 10^6 and 7.5% (v/v) DMSO. The time from the mixing of the cell preparation and DMSO stock solution to the start of the freezing process should be no less than 15 min and no longer 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 vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. If

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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.

7. The frozen preparations are stored in either the vapor or liquid phase of a nitrogen refrigerator.
8. To establish a culture from the frozen state place an ampule in a water bath set at 35°C. Immerse the vial just sufficiently to cover only 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 inoculate into a fresh tube or flask of ATCC medium 1034.
10. Incubate at 35°C with the cap screwed on tightly (incubate a test tube on a 15° horizontal slant).

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.

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.

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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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