ATCC

Product Sheet

Prototheca wickerhamii (ATCC® 16529™)

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: *Prototheca wickerhamii* (ATCC® 16529™)

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: NRRL YB-4330 [UTEX 1553]
Deposited Name: *Prototheca wickerhamii* Tubaki and Soneda
Depositor: WB Cooke
Isolation: Household plumbing, Peoria, IL, (?)

Propagation

Growth Conditions
Temperature: 20°C to 25°C
Culture System: Axenic

Medium
ATCC® Medium 28: Emmons' modification of Sabouraud's agar

Instructions for Complete Medium
ATCC Medium 28

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 to 3 min). Immerse the ampule enough to cover only the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically transfer the entire contents to a single 16 x 125 mm screw-capped test tube containing a slant of ATCC Medium 28 or distribute over the surface of an agar plate of ATCC medium 28.
3. If a test tube culture has been established, incubate upright at 20°C to 25°C with the cap screwed on loosely. If a plate culture has been established, wrap the plate with parafilm and incubate inverted at 20°C to 25°C.

Culture Maintenance

1. Transfer cells with an inoculating loop to a tube or plate of fresh agar medium from a growing culture at or near peak density.
2. Incubate as described in step 3 under the section for establishing a culture.

Cryopreservation

Harvest and Preservation

1. Harvest cells from a culture which is at or near peak density by adding 3.0 mL to 5.0 mL fresh ATCC medium 28 broth to the slant or plate and washing cells into suspension. It may be helpful to rub the surface of the agar with a spread bar or inoculating loop to detach adhering cells.
2. Adjust the concentration of cells to 2 x 10^7 mL with fresh broth medium, then dilute to half this concentration by adding an equal amount of a 20% (v/v) sterile solution of either DMSO or glycerol in fresh ATCC medium 28 broth.
3. Dispense in 0.5 mL aliquots into 1.0 mL to 2.0 mL sterile plastic screw-capped cryules (special plastic vials for cryopreservation). The time from mixing of the cell preparation and the cryoprotective solution to the start of the cooling cycle should be no less than 15 min and no greater than 30 min.
4. 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.)
5. 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 can be stored

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between -80°C and -70°C for no longer than one week.
6. To establish a culture from the frozen state place an ampule in a water bath set at 35°C until thawed (2 to 3 min). Immerse the ampule enough to cover only the frozen material. Do not agitate the ampule.
7. Immediately after thawing, do not leave in the water bath, aseptically remove the contents of the ampule and add to a fresh slant of ATCC medium 28 or the surface of an agar plate of ATCC medium 28.
8. Maintain as described above.

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.

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.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

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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