



Product Sheet

# *Prototheca zopfii* (ATCC®) 16532™)

Please read this **FIRST**



## 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 zopfii* (ATCC® 16532™)

American Type Culture Collection  
PO Box 1549  
Manassas, VA 20108 USA  
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## Description

**Strain Designation:** PR-30 [UTEX 1438]

**Deposited Name:** *Prototheca zopfii* Kruger

**Depositor:** WB Cooke

**Isolation:**

slime flux of locust tree, Peoria, IL, (?)

## Propagation

### Growth Conditions

**Temperature:** 25.0°C

Duration: axenic

**Protocol:** ATCCNO: 16522 SPEC: This strain is distributed as a freeze-dried preparation. See the general procedures for opening a freeze-dried vial. Aseptically add 0.5 ml of ice cold medium to containing 12% sucrose to the freeze-dried inner shell vial. Once completely rehydrated, aseptically transfer the material to a 100 mm agar plate of the appropriate medium and evenly distribute the material over the surface of the agar with a spread bar. Subculture 4-6 weeks when incubated at 25C. Subculture 6-12 months when incubated at 18C To subculture, transfer a loopful of material to a fresh plate and spread evenly over the surface.

### Medium

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

### Instructions for Complete Medium

ATCC Medium 28

### Culture Maintenance

1. For a slant culture, transfer cells with an inoculating loop to a tube of fresh agar slant from a growing culture at or near peak density.
2. For a plate culture, transfer cells with an inoculating loop to a fresh agar plate a growing culture at or near peak density.
3. Incubate as described in steps 3 or 4 above under the heading, establishing a culture from a freeze-dried vial.

## Cryopreservation

### CRYOPRESERVATION

1. Harvest cells from a culture which is at or near peak density by adding 3.0 ml fresh ATCC medium 28 broth to the slant and washing cells into suspension.
2. Adjust the concentration of cells to  $4 \times 10^9$ /ml with fresh broth medium, then dilute to half this concentration by adding an equal amount of a 20% (v/v) sterile DMSO solution in fresh ATCC medium 28 broth.
3. Dispense in 0.5 ml aliquots into 1.0 - 2.0 ml sterile plastic screw-capped cryules (special plastic vials for cryopreservation). The time from mixing of the cell preparation and the methanol solution, before the cooling cycle begins, should be no greater than 15 min.
4. Place vials in a controlled rate freezing unit. From room temperature cool at  $-1^\circ\text{C}/\text{min}$  to  $-40^\circ\text{C}$ . If freezing unit can compensate for the heat of fusion, maintain rate at  $-1^\circ\text{C}/\text{min}$  through heat of fusion. At  $-40^\circ\text{C}$  plunge ampules into liquid nitrogen. Alternatively, place the vials in a Nalgene  $1^\circ\text{C}$  freezing apparatus. Place the apparatus at  $-80^\circ\text{C}$  for 1.5 to 2 hours and then plunge ampules into liquid nitrogen. (The cooling rate in this apparatus is approximately  $-1^\circ\text{C}/\text{min}$ .)
5. The frozen preparations should be stored in either the vapor or liquid phase of a nitrogen refrigerator. Frozen preparations stored below  $-130^\circ\text{C}$  are stable indefinitely. Those stored at temperatures above  $-130^\circ\text{C}$  are progressively less stable as the storage temperature is elevated. Vials can be stored between  $-80$  and  $-70^\circ\text{C}$  for no longer than one week.
6. To establish a culture from the frozen state place an ampule in a water bath set at  $35^\circ\text{C}$ . Immerse the vial to a level just above the surface of the frozen material. Do not agitate the vial.
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



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## 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

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## Disclaimers

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

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Additional information on this culture is available on the ATCC web site at [www.atcc.org](http://www.atcc.org).

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