




Product Sheet


# *Cryptodiffugia operculata* (ATCC® PRA-365™)

Please read this FIRST



Storage Temp.  
**Frozen: -70°C or colder**  
**Freeze-Dried: 2°C to 8°C**  
**Live Culture: See Protocols Section**

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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: *Cryptodiffugia operculata* (ATCC® PRA-365™)

American Type Culture Collection  
PO Box 1549  
Manassas, VA 20108 USA  
[www.atcc.org](http://www.atcc.org)

800.638.6597 or 703.365.2700  
Fax: 703.365.2750  
Email: [Tech@atcc.org](mailto:Tech@atcc.org)

Or contact your local distributor

## Description

**Strain Designation:** Smith College  
**Deposited Name:** *Cryptodiffugia operculata*  
**Depositor:** DJG Lahr  
**Isolation:** Mixed protozoa culture, Carolina Biological

## Propagation

### Growth Conditions

**Temperature:** 20°C to 25°C

**Atmosphere:** Aerobic

**Culture system:** Xenic, with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™) as a food source

### Medium

ATCC® Medium 802: Sonneborn's Paramecium medium

ATCC® Medium 2348: Freshwater Diplophrys medium

### Instructions for Complete Medium

ATCC Medium 802 and ATCC Medium 2348, combined in equal parts and inoculated with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™)

## 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 ampules 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 such that the lip of the ampule remains above the water line. Thawing time is approximately 2 to 3 minutes. Do not agitate the ampule. Do not leave ampule in water bath after it is thawed.
2. Add the thawed contents to a T-25 flask containing 10 mL complete medium bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™).
3. Incubate with the cap tightly sealed at 20-25°C.

### Culture Maintenance

Subculture every 7-14d to a fresh T-25 flask of complete, bacterized medium in the following manner:

1. Vigorously agitate the flask (or scrape the flask bottom using a sterile cell scraper) and aseptically transfer 0.5 mL to 1.0 mL from a growing culture to a T-25 tissue culture flask containing 10 mL complete medium bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™).
2. Incubate with the cap tightly sealed at 20-25°C.

## Cryopreservation

### Reagents

#### Cryoprotective Solution

DMSO, 1.5 mL

Fresh growth medium w/o bacteria, 8.5 mL

### Harvest and Preservation


1. Mix the components of the cryoprotective solution 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 that is at or near peak density by filtration and centrifugation at 400 x g for 5 min.
3. Adjust the concentration of cells to at least 2 x 10<sup>5</sup>/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 cryovials (special plastic vials)



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- for cryopreservation).
- 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.)
  - Ampules are stored in either the vapor or liquid phase of a nitrogen refrigerator.
  - 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, do not leave in water bath, aseptically remove the contents of the ampule and inoculate into a T-25 tissue culture flask containing 10 mL complete medium bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™).
  - Incubate at 20-25°C with the cap screwed on tightly.
  - Follow the protocol for maintenance of culture.



### References

References and other information relating to this product are available online at [www.atcc.org](http://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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Additional information on this culture is available on the ATCC web site at [www.atcc.org](http://www.atcc.org).

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