



Product Sheet

# *Naegleria clarki* (ATCC®) 30544™)

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: *Naegleria clarki* (ATCC® 30544™)

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:** PD  
**Deposited Name:** *Naegleria gruberi* Schardinger  
**Depositor:** WD Odell  
**Isolation:**  
sewage effluent, Ohio, 1969

## Propagation

### Growth Conditions

**Temperature:** 25.0°C

Duration: grown with *Escherichia coli* ATCC 11775

**Protocol:** ATCCNO: 30133 SPEC: This strain is shipped as a dried preparation. Aseptically add 1 ml of sterile distilled water to the inner vial, remove the filter paper pellet with a pair of forceps, and place it in the center of an agar plate of ATCC medium 711 or 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<sup>2</sup>) 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 every 3-4 wks (or at 10-14 d intervals if cultured at 35°C).

Note: a monoxenic amoeba culture can be established in this manner using any suitable bacterial food source.

## Cryopreservation

### Cryoprotective Solution

DMSO	1.5 ml
Page's Balanced Salt 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 1323 (Page's Balanced Salt 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<sup>6</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 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.
10. Follow the protocol for maintenance of culture.



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## 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

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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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