



Product Sheet

# *Vannella miroides* (ATCC®) 30945™)

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: *Vannella miroides* (ATCC® 30945™)

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

**Strain Designation:** F-49

**Deposited Name:** *Vannella* sp.

**Depositor:** JL Griffin

**Isolation:**

aquarium, Room 215, Anatomy Dept., Harvard Med. Sch., 1963

## Propagation

### Growth Conditions

**Temperature:** 25.0°C

Duration: grown with mixed bacteria

### Medium

ATCC® Medium 997: Fresh water amoeba medium

## Instructions for Complete Medium

ATCC medium 997; grown with mixed bacteria

## Culture Maintenance

1. Streak an ATCC medium 997 plate with *Klebsiella pneumoniae* (ATCC® 700831) 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 at 10-14 d intervals.

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

## Cryopreservation

1. Harvest cells from a culture which is at or near peak density by adding 5 ml fresh 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 amoebae.
2. Transfer the liquid medium to a sterile centrifuge tube.
3. If the cell concentration does not exceed  $2 \times 10^6$  cells/ml adjust the suspension to that concentration. To adjust the concentration, centrifuge at 600 x g for 5 min and resuspend the pellet in the volume of fresh medium required to yield  $2 \times 10^6$ .
4. While cells are centrifuging prepare a 15% (v/v) solution of sterile DMSO as follows: Add the required volume of DMSO to a glass screw-capped test tube and place it in an ice bath. Allow the DMSO to solidify. Add the required volume of refrigerated medium. Dissolve the DMSO by inverting the tube several times.  
\*NOTE: If the DMSO solution is not prepared on ice, an exothermic reaction will occur that may precipitate certain components of the medium.
5. Mix the cell preparation and the DMSO in equal portions. Thus, the final concentration will be at least  $10^6$  cells/ml and 7.5% (v/v) DMSO. The equilibration time (the time between addition of DMSO and the start of the cooling cycle) should be no less than 15 min and no longer than 60 min.
6. Dispense in 0.5 ml aliquots into 1.0 - 2.0 ml sterile plastic screw-capped cryules (special plastic vials for cryopreservation).
7. 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.
8. The frozen preparations are stored in either the vapor or liquid phase of a nitrogen freezer.
9. To establish a culture from the frozen state place an ampule in a water bath set at 35°C (2-3 min). Immerse the vial to a level just above the surface of the frozen material. Do not agitate the vial.
10. Immediately after thawing, aseptically remove the contents of the ampule and distribute to the center of a fresh plate of ATCC medium 997. Distribute the material evenly over the plate using a spread bar. Incubate at 25°C.

## References

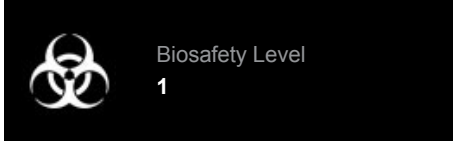
References and other information relating to this product are available online at [www.atcc.org](http://www.atcc.org).



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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.

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## **Disclaimers**

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