ATCC

Product Sheet

# *Vannella miroides* (ATCC® 30945™)

**Please read this FIRST**

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: *Vannella miroides* (ATCC® 30945™)

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:** 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 ameba 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 $mm^2$ ) 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 x $10^6$ 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 x $10^6$.
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.

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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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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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