



Product Sheet

# *Sawyeria marylandensis* (ATCC® 50653™)

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: *Sawyeria marylandensis* (ATCC® 50653™)

American Type Culture Collection  
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Manassas, VA 20108 USA  
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Fax: 703.365.2750  
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## Description

**Strain Designation:** GF-1

**Deposited Name:** *Sawyeria marylandensis* O'Kelly et al.

**Depositor:** TA Nerad

**Isolation:**

iron rich small stream near Great Falls, MD, 1994

## Propagation

### Growth Conditions

**Temperature:** 25.0°C

Duration: Anaerobic, xenic, grown with *Klebsiella pneumoniae* subsp. *pneumoniae* ATCC 700831 as a food source. Cultivated in 13 ml volumes in tightly capped 16 x 125 mm screw-capped test tubes and incubated on a 15 degree horizontal slant.

### Medium

ATCC® Medium 802: Sonneborn's Paramecium medium

## Instructions for Complete Medium

ATCC Medium 802

### Culture Maintenance

1. Prepare bacterized ATCC medium 802 i.e., inoculate 2 16 x 125 mm screw-capped test tubes containing 12 ml of medium with a bacteriological loop of *Enterobacter aerogenes* (ATCC® 13048) from a nutrient agar slant (ATCC medium 3). Incubate overnight with caps screwed on tightly.
2. When the culture is at or near peak density, invert the test tube 10 times and aseptically transfer 0.25 ml of culture to a fresh test tube containing 12 ml bacterized ATCC medium 802.
3. Screw the caps on tightly and incubate at 25°C.

## Cryopreservation

1. Harvest the cells from a culture that is at or near peak density by centrifuging at 850 x g for 5 minutes.
2. If the cell concentration exceeds the required level do not centrifuge, but adjust the concentration to between  $2 \times 10^6$  and  $2 \times 10^7$  cysts/ml with fresh medium. If the concentration is too low, centrifuge at 850 x g for 5 min and resuspend the pellet in the volume of fresh medium required to yield the desired concentration.
3. While cells are centrifuging prepare a 20% (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.
4. Mix the cell preparation and the DMSO in equal portions. Thus, the final concentration will be between  $10^6$  and  $10^7$  cells/ml and 10% (v/v) DMSO. The time from the mixing of the cell preparation and DMSO stock solution before the freezing process is begun should be no less than 15 min and no longer than 30 min.
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 the vials in a controlled rate freezing unit. From room temperature cool at  $-1^\circ\text{C}/\text{min}$  to  $-40^\circ\text{C}$ . If the freezing unit can compensate for the heat of fusion, maintain rate at  $-1^\circ\text{C}/\text{min}$  through the heat of fusion. At  $-40^\circ\text{C}$  plunge 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  $-1^\circ\text{C}/\text{min}$ .)
7. The frozen preparations are stored in either the vapor or liquid phase of a nitrogen freezer.
8. To establish a culture from the frozen state place an ampule in a water bath set at  $35^\circ\text{C}$  (2-3 min). Immerse the vial just sufficient to cover the frozen material. Do not agitate the vial.
9. Immediately after thawing, aseptically transfer contents to a 16 x 125 mm screw-capped test tube containing 12 ml of bacterized ATCC Medium 802. Incubate the culture on a  $15^\circ$  horizontal slant at  $25^\circ\text{C}$ .

## 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.

### 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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