Sawyeria marylandensis O'Kelly et al.

50653™

Description

Strain designation: GF-1
Deposited As: *Sawyeria marylandensis* O'Kelly et al.
Type strain: Yes

Storage Conditions

Product format: Test tube

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always

used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Medium:
ATCC Medium 802: Sonneborn's Paramecium medium
Instructions for complete medium: ATCC Medium 802
Temperature: 25°C
Atmosphere: Anaerobic
Culture system: Xenic
Incubation: 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.

Handling Procedures

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 x 10^6 and 2 x 10^7cysts/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°C/min to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through the heat of fusion. At -40°C plunge 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. 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°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° horizontal slant at 25°C.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Sawyeria marylandensis* O'Kelly et al. (ATCC 50653)

References

References and other information relating to this material are available at www.atcc.org.

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