Spironucleus barkhanus Sterud et al.

50467™

Description

Strain designation: Tt-1
Deposited As: *Spironucleus barkhanus* Sterud et al.
Type strain: No

Storage Conditions

Product format: Frozen

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

Temperature: 15-18°C
Atmosphere: Anaerobic
Culture system: Axenic

Handling Procedures

Frozen ampules packed in dry ice should either be thawed immediately or stored in liquid nitrogen. If liquid nitrogen storage facilities are not available, frozen ampules may be stored at or below -70°C for approximately one week. **Do not under any circumstance store frozen ampules at refrigerator freezer temperatures (generally -20°C).** Storage of frozen material at this temperature will result in the death of the culture.

1. To thaw a frozen ampule, place it in a 35°C water bath , until thawed (2-3 min).

Immerse the ampule just sufficient to cover the frozen material. Do not agitate the ampule.

2. Immediately after thawing, aseptically transfer contents to a screw-capped test tube containing 13 ml ATCC Medium 2695 cooled to a temperature not above 18°C. Incubate the tube on a 15° horizontal slant at 15-18°C.

Culture maintenance:

1. When the culture has reached or is near peak density, place the tubes on ice for 10 minutes.

2. Gently invert the culture tube 10 times and aseptically transfer a 0.1-0.4 ml aliquot to a screw-capped test tube containing 13 ml ATCC Medium 2695 cooled to a temperature not above 18°C.

3. Incubate the culture on a 15° horizontal slant at 15-18°C.

4. Transfer the culture every 3-4 days as described in steps 1-2. The transfer interval will depend on the size of the inoculum and the quality of the medium. This should be empirically determined by examining the culture on a daily basis until the growth cycle has stabilized. Do not allow the culture to overgrow. The culture will crash soon after reaching peak density.

Cryopreservation:

1. Harvest cells from a culture that is at or near peak density. To detach cells from the wall of the culture tubes place on ice for 10 minutes. Invert tubes several times until the majority of the cells are in suspension. Centrifuge tubes at 800 x g for 5 minutes.

2. Adjust the concentration of cells to 2 x 10^7/ml in fresh medium.

3. Before centrifuging prepare a 24% (v/v) solution of sterile DMSO in fresh medium containing 8% (w/v) sucrose. The solution is prepared as follows:

a) Add 10.5 g sucrose to 10 ml of fresh medium and filter sterilize through a 0.2 mm filter;

b) Add 2.4 ml of DMSO to an ice cold 20 x 150 mm screw-capped test tube;

c) Place the tube on ice and allow the DMSO to solidify (~5 min) and then add 7.6 ml

of ice cold medium prepared in step 3a. The final concentration will be 24% (v/v) DMSO and 8% (w/v) sucrose;

d) Invert several times to dissolve the DMSO;

e) Allow to warm to room temperature.

4. Mix the cell preparation and the cryoprotective agent, prepared in step 3, in equal portions. Thus, the final concentration will equal 12% (v/v) DMSO + 4% sucrose (w/v) and 10^7 cells/ml. 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 should be stored in either the vapor or liquid phase of a nitrogen refrigerator. Frozen preparations stored below -130°C are stabile indefinitely. Those stored at temperatures above -130°C are progressively less stabile as the storage temperature is elevated.

8. To establish a culture from the frozen state place an ampule in a water bath set at 35°C. Immerse the vial just to a level just above the surface of the frozen material. Do not agitate the vial.

9. Immediately after thawing, do not leave in the water bath, aseptically remove the contents of the ampule and inoculate a 16 x 125 mm screw-capped test tube containing 13 ml ATCC Medium 2695 cooled to a temperature not above 18°C.

10. Incubate the culture on a 15° horizontal slant at 15-18°C.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Spironucleus barkhanus* Sterud et al. (ATCC 50467)

References

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

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Revision

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