Spironucleus sp.

50632™

Description

Strain designation: RFT
Deposited As: *Spironucleus* sp.
Type strain: No

Storage Conditions

Product format: Frozen
Storage conditions: -80°C or colder for 1 week, vapor phase of liquid nitrogen for long-term storage

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 1671: Blastocystis egg medium
Temperature: 25°C
Atmosphere: Anaerobic

Handling Procedures

Storage and Culture Initiation
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. If the HIHS has not already been added, add 1.5 mL to each tube.
2. Loosen caps one full turn and place tubes in an anaerobic jar. Add a BD GasPak (one anaerobic system GasPak per anaerobic culture jar). Close the vessel securely and incubate at 25°C for at least 48 hours. If the GasPak is of the variety that makes use of a palladium catalyst, the catalyst should be replaced biweekly.
3. Thaw the frozen ampule in a 35°C water bath without agitation until all of the contents are liquid (about 2-3 minutes).
4. Aseptically and gently, lower a sterile Pasteur pipette from which the air has been expelled to the bottom of the liquid in the ampule and slowly aspirate the entire contents into the pipette. Be careful to minimize agitation of the fluid and so not introduce air bubbles from the tip of the pipette.
5. Inoculate a fresh tube of previously-reduced ATCC medium 1671 by inserting the Pasteur pipette tip aseptically through the liquid overlay-air interface (avoid expulsion of air bubbles or culture) and moving the tip of the pipette to the base of the solid-overlay interface. Expel the entire contents of the Pasteur pipette into the culture tube (again avoid expulsion of air bubbles), then tighten the cap immediately unless placing the tube directly into an anaerobic jar.
6. With the cap of the freshly inoculated test tube loosened one full turn, place it in an anaerobic jar containing a BD GasPak and incubate at 25°C.

Culture maintenance:

1. When the culture has reached or is near peak density, remove the growing culture without agitation from the anaerobic jar and immediately screw the tube cap(s) down tightly.
2. The strain grows at the bottom of the liquid overlay as a dense mass of cells. Carefully introduce a sterile Pasteur pipette aseptically through the liquid overlay-air interface (avoid expulsion of air bubbles) and move the tip of the pipette to the cell mass at the base of the solid-overlay interface. Aspirate approximately one third of the mass into the pipette. After removing the pipette, tighten the cap immediately unless placing the tube directly into an anaerobic jar.
3. Inoculate a fresh tube of previously-reduced ATCC medium 1671 as described above. Place the freshly inoculated tube into the anaerobic jar with the cap loosened one full turn, add a GasPak, and quickly seal the jar. Incubate at 25°C.
4. Subculture every 7-14 days or as necessary. An optimal transfer interval should be empirically determined by examining the culture on a daily basis until the

growth cycle has stabilized.

Note: Addition of antibiotics to the culture may be necessary if the bacterial density in the culture begins to inhibit growth of *Spironucleus*. The optimal antibiotic treatment regimen should be empirically determined using a test culture kept in parallel to the original to avoid inadvertently harming *Spironucleus*.

Cryopreservation:

1. Harvest cells from a culture that is at or near peak density by centrifugation at 800 x g for 5 min. The cells grown in a medium containing agar are concentrated by centrifugation, a solid pellet does not form. The soft pellet is resuspended to desired cell concentration with agar-free supernatant.

2. Adjust the concentration of cells to 2 x 10^6 - 10^7/ml in fresh medium.

3. While cells are centrifuging prepare a 10% (v/v) solution of sterile DMSO in fresh medium.

a) Add 1.0 ml of DMSO to an ice cold 20 x 150 mm screw-capped test tube;

b) Place the tube on ice and allow the DMSO to solidify (~5 min) and then add 9.0 ml of ice cold medium;

c) Invert several times to dissolve the DMSO;

d) Allow to warm to room temperature.

4. Mix the cell preparation and the DMSO in equal portions. Thus, the final concentration will be 10^6 - 10^7 cells/ml and 5% (v/v) DMSO. The time from the mixing of the cell preparation and DMSO stock solution before the freezing process is begun should 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. Vials should not be stored above -55°C.

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 mls of ATCC medium 1978 adjusted to pH 6.0.

10. Incubate the culture on a 15° horizontal slant at 35°C.

Notes

This xenic culture contains the original bacterial flora present when the parasite was first isolated.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Spironucleus* sp. (ATCC 50632)

References

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

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