ATCC

Product Sheet

Spironucleus sp. (ATCC® 50632™)

Please read this FIRST

Storage Temp.
Frozen Cultures:
-70°C for 1 week; liquid N_2 vapor for long term storage

Freeze-dried Cultures:
2-8°C

Live Cultures:
See Protocols section for handling information

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: *Spironucleus sp.* (ATCC® 50632™)

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: RFT
Deposited Name: *Spironucleus* sp.
Depositor: CH Zierdt
Isolation: Not applicable

Notes

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

Propagation

Growth Conditions
Temperature: 25°C
Atmosphere: Anaerobic

Medium
ATCC® Medium 1671: Blastocystis egg medium

Protocols

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*.

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Cryopreservation

Harvest and Preservation

1. Two to three days in advance, prepare fresh tubes containing ATCC medium 1671 and 25% HIHS in an anaerobic jar with tube caps loosened one full turn. 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.
2. Using the liquid overlay from the previously-reduced tubes of medium prepared in step 1 above, prepare a 20% (v/v) sterile DMSO solution in Stone's Modification of Locke's Solution in the following manner:
   a. Add the required volume of DMSO to a 20 x 150 mm screw-capped test tube;
   b. Place the tube on ice and allow the DMSO to solidify (~5 min), then add the required volume of previously-reduced liquid overlay. Chemical heat will be liberated from this combination so allow the solution to cool to room temperature;
   c. If time allows, loosen the tube cap one full turn and place in an anaerobic jar with an anaerobic GasPak for at least 48 hours prior to use.
3. When the test tube cultures are at or near peak density remove the tubes from the anaerobic jar and immediately screw the caps on tightly. Opening one tube at a time, gently remove the cells from the bottom of the egg medium slants and pool in a single 16 x 125 mm screw-capped test tube (work quickly to avoid prolonged exposure to air).
4. Adjust the cell concentration to 2 x 10^6 - 2 x 10^7 cells/mL using overlay from a reduced tube of medium. If the concentration of cells is too low centrifuge at 500 x g for 5 minutes. Adjust the volume of supernatant to yield the desired final cell concentration.
5. Mix the cell preparation and the cryoprotective agent prepared in step 2 in equal portions. Thus, the final concentration will equal 10% (v/v) DMSO and 10^6 - 10^7 cells/mL. The time from the mixing of the cell preparation and DMSO stock solution to the start of the freezing process should be no less than 15 min and no longer than 30 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 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.)
8. 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.
9. Before thawing an ampule do the following: Place tubes containing ATCC medium 1671 and 25% HIHS in an anaerobic jar with tube caps loosened one full turn. 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.
10. Thaw the frozen ampule in a 35°C water bath without agitation until all of the contents are liquid (about 2-3 minutes).
11. 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.
12. 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.
13. 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.
14. Follow the procedure for maintenance of the culture as described above.

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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