



***Blastocystis* sp.**

50578™

Description

Strain designation: NIH:1295:1

Deposited As: *Blastocystis* sp.

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 2

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

Instructions for complete medium: ATCC Medium 1671

Temperature: 35°C

Atmosphere: Anaerobic

Incubation: Monoprotist cultures grown with mixed bacteria

Handling Procedures

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 caps 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, aspirate approximately one third of the mass into the pipette. Tighten the cap immediately unless placing the tube directly into an anaerobic jar.

3. Inoculate a fresh tube of reduced ATCC medium 1671. Place the freshly inoculated tube into the anaerobic jar with the caps loosened one full turn, prepare the GasPak, and quickly seal the jar. Incubate at 35°C.

4. Subculture every 2-3 days.

Cryopreservation:

1. Two to three days in advance, prepare a 14% (v/v) sterile glycerol plus 14% (v/v) sterile DMSO solution in Stone's Modification of Locke's Solution in the following manner:

a) Combine 0.84 ml of sterile glycerol and 0.84 ml of sterile DMSO in a 16 x 125 mm screw-capped test tube. Chemical heat will be liberated from this combination so allow the solution to cool to room temperature.

b) To the glycerol/DMSO solution add 4.32 ml of Stone's Modification of Locke's Solution. Mix by inverting several times.

c) Loosen the cap one full turn and place in an anaerobic jar with an anaerobic GasPak for 2-3 days.

2. 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. One by one 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).

3. Adjust the concentration to $1.0-2.0 \times 10^7$ 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.

4. Mix the cell preparation and the cryoprotective agent, prepared in step 1, in equal portions. Thus, the final concentration will equal 7% (v/v) glycerol, 7% (v/v) DMSO and $5.0 \times 10^6 - 1.0 \times 10^7$ 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 cryoles (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°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°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

$-1^{\circ}\text{C}/\text{min}$.)

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 stable indefinitely. Those stored at temperatures above -130°C are progressively less stable as the storage temperature is elevated.

8. Before thawing an ampule do the following. Loosen caps one full turn and place tubes containing ATCC medium 1671 and 25% HHS in an anaerobic jar. Add a BBL GasPak (one anaerobic system GasPak per BBL GasPak 100 anaerobic culture jar). Close the vessel securely and incubate at 35°C for at least 48 hours. The palladium catalyst in the GasPak jar should be replaced biweekly with fresh catalyst.

9. Thaw the frozen ampule in a 35°C water bath without agitation until all of the contents are liquid (about 2-3 minutes).

10. 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.

11. With the cap of the test tube loosened one full turn place it in an anaerobic jar containing a BBL GasPak and incubate at 35°C for at least 48 hours.

12. Subculture every 2-3 days.
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Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Blastocystis* sp. (ATCC 50578)

References

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

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