ATCC

Product Sheet

Blastocystis hominis (ATCC® 50614™)

Please read this FIRST

Biosafety Level
2

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: *Blastocystis hominis* (ATCC® 50614™)

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: WBY
Deposited Name: *Blastocystis hominis* Brumpt
Depositor: CH Zierdt
Isolation:

Propagation

Growth Conditions
Temperature: 25.0°C
Duration: anaerobic
Protocol: ATCCNO: 50177 SPEC: The following directions for recovery from the frozen preparation must be carefully followed if a culture is to be successfully established: 1. Upon receipt, it is necessary to store the frozen ampule at temperatures of -135C or lower for at least 48 hours prior to thawing. The ampule may be stored in a mechanical refrigerator set at -60 to -70C if the storage time does not exceed 48 hours. The ampule should be removed from the dry-ice when stored in either a mechanical or nitrogen refrigerator. 2. After the frozen ampule is placed in storage, prepare a single tube of reduced ATCC medium 1671 in the manner outlined in step 1 under Maintenance of Strain and Subculturing below. 3. When the medium is ready for inoculation, thaw the ampule in a 35C water bath without agitation (do not fully immerse ampule) and remove vial immediately after the preparation has become completely liquid. 4. Aseptically, 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 do not introduce air bubbles at any time. These organisms are sensitive to oxygen. 5. Add the cell suspension to the bottom of the liquid overlay of the reduced tube of ATCC medium 1671. Avoid expulsion of air bubbles from the tip of the pipette. 6. With the cap of the test tube loosened (1 full turn) place it in an anaerobic jar which contains a BBL GasPak (one anaerobic system GasPak per BBL GasPak 100 anaerobic culture jar). Close the vessel securely and incubate at 35C. Maintenance of Strain and Subculturing: Subculture at 2-3 day intervals. 1. Prior to inoculation, reduce (lower redox potential) complete ATCC medium 1671 by placing the tubes with caps on loosely (1 full turn) in an anaerobic jar containing a BBL GasPak (one anaerobic system GasPak per BBL GasPak 100 anaerobic culture jar) for at least 48 hours. Note: The palladium catalyst in the GasPak jar should be replaced biweekly with fresh catalyst. 2. To subculture, remove the growing cultures without agitation from the anaerobic jar and immediately screw the caps down tightly. The strain grows at the bottom of the liquid overlay as a dense mass of cells. 3. 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 the culture is to be promptly placed in the anaerobic jar. 4. Place the freshly inoculated culture into the anaerobic jar with the cap loosened (1 full turn), prepare the GasPak and quickly seal the jar.

Medium
ATCC® Medium 1671: Blastocystis egg medium

Instructions for Complete Medium
ATCC Medium 1671

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 25°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.

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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 x 10^7cells/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 x 10^6 - 1.0 x 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. Before thawing an ampule do the following. Loosen caps one full turn and place tubes containing ATCC medium 1671 and 25% HIHS 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 25°C for at least 48 hours.
12. Subculture every 2-3 days.

References

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

Biosafety Level: 2

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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