Blastocystis hominis Brumpt

50177™

Description

Blastocystis hominis strain Nand II is a parasitic protozoan that was isolated in 1979 from a human in Bethesda, Maryland. This product has applications in enteric disease research.

Strain designation: Nand II
Deposited As: *Blastocystis hominis* Brumpt
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 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
Temperature: 35°C
Atmosphere: Anaerobic
Culture system: Axenic

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 35°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 35°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 35°C.
4. Subculture every 2-3 days.

Cryopreservation:

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 35°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.
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. 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).
3. Adjust the cell concentration to 1.0-2.0 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.
4. 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 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. Vials should not be stored above -55°C.
8. 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 35°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.
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. 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.
12. 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 35°C.
13. Subculture every 2-3 days.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Blastocystis hominis* Brumpt (ATCC 50177)

References

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

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