ATCC

Product Sheet

Hexamita inflata (ATCC® 50268™)

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: *Hexamita inflata* (ATCC® 50268™)

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: AZ-4
Deposited Name: *Hexamita* sp.
Depositor: TK Sawyer
Isolation: Fibrous plant roots and soil from small pond, Desert Museum, Tucson, AZ, 1991

Notes

This strain is cultivated with a non-pathogenic strain of *K. pneumoniae* or *E. aerogenes* as a food source. Other unidentified bacteria are also present.

Propagation

Growth Conditions
Temperature: 25°C
Atmosphere: Microaerophilic

Medium
ATCC® Medium 1773: Hexamita medium

Instructions for Complete Medium
Media: ATCC Medium 1773 inoculated with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™).

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 ampoules 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. One day before thawing the ampule, prepare bacterized ATCC medium 1773, i.e. inoculate medium with a bacteriological loop of *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™) from a nutrient agar slant (ATCC medium 3).
2. To thaw a frozen ampule, place it in a 35°C water bath such that the lip of the ampule remains above the water line. Thawing time is approximately 2 to 3 minutes. Do not agitate the ampule. Do not leave ampule in water bath after it is thawed.
3. Immediately after thawing, aseptically transfer contents to a 16 x 125 mm screw-capped test tube containing 12 mL of ATCC Medium 1773. Screw the cap on tightly and incubate on a 15° horizontal slant at 25°C.

Culture Maintenance

1. Prepare bacterized ATCC medium 1773.
2. When the culture is at or near peak density, rub the surface of the tube with a sterile cotton swab, and agitate the swab to dislodge the adherent cells. Invert gently 10 times to distribute cells evenly.
3. Transfer approximately 0.25 mL to a 16 x 125 mm screw-capped test tube containing 12 mL of fresh ATCC medium 1773.
4. Screw the cap on tightly and incubate on a 15° horizontal slant at 25°C.

Cryopreservation

Harvest and Preservation

1. Harvest the cells from a culture that is at or near peak density by centrifuging at 850 x g for 5 minutes.
2. If the cell concentration exceeds the required level do not centrifuge, but adjust the concentration to between 2 x 10^6 and 2 x 10^7 cells/mL with fresh medium. If the concentration is too low, centrifuge at 850 x g for 5 min and resuspend the pellet in the volume of fresh medium required to yield the desired concentration.
3. While cells are centrifuging prepare a 20% (v/v) solution of sterile DMSO as follows: Add the required volume of DMSO to a glass screw-capped test tube and place it in an ice bath. Allow the DMSO to

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solidify. Add the required volume of refrigerated medium. Dissolve the DMSO by inverting the tube several times.
*NOTE: If the DMSO solution is not prepared on ice, an exothermic reaction will occur that may precipitate certain components of the medium.

4. Mix the cell preparation and the DMSO in equal portions. Thus, the final concentration will be between 10^6 and 10^7 cells/mL and 10% (v/v) DMSO. 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 vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. If freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through heat of fusion. At -40°C plunge ampules 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 are stored in either the vapor or liquid phase of a nitrogen freezer.
8. To establish a culture from the frozen state place an ampule in a water bath set at 35°C (2-3 min). Immerse the vial just sufficient to cover the frozen material. Do not agitate the vial.
9. Immediately after thawing, aseptically remove the contents of the ampule and inoculate into 12 mL of fresh ATCC medium 1773 in a 16 x 125 screw-capped test tube. Incubate on a 15° horizontal slant at 25°C.

References

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

Biosafety Level: 1

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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