ATCC

Product Sheet

Tetrahymena sp. (ATCC® PRA-370™)

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: *Tetrahymena sp.* (ATCC® PRA-370™)

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: North India strain BP 610
Deposited Name: *Chilodonella uncinata*
Depositor: B Das
Isolation: Mosquito larvae, Delhi, North India, 1999

Propagation

Growth Conditions
Temperature: 20°C to 25°C
Atmosphere: Aerobic
Culture system: With *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™).

Medium
ATCC® Medium 802: Sonneborn's Paramecium medium

Instructions for Complete Medium
ATCC Medium 802 inoculated with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™).

Protocols

Handling of Live Culture
This strain is routinely shipped as a growing culture in a glass 16 x 125 mm screw-capped test tube. The volume of the cell suspension is approximately 5 mL. When the culture arrives remove it promptly from the shipping container. **Do not store the culture at refrigeration temperatures before handling.** To assure viability, immediately loosen the test tube cap and incubate upright at 25°C for at least one hour before observing the culture. There should be numerous active trophozoites in suspension. If the numbers are low the culture may have been exposed to temperature extremes in transit. Regardless of the state of the culture, aseptically transfer a 0.5 mL aliquot to a T-25 flask containing 10 mL of ATCC medium 802 bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™). Incubate with the cap tightly sealed at 25°C.

Culture Maintenance
Subculture every seven days to a fresh T-25 flask of bacterized medium in the following manner:

1. Vigorously agitate the flask and aseptically transfer 0.5 mL from a growing culture to a T-25 tissue culture flask containing 10.0 mL of ATCC medium 802 bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™).
2. Incubate with the cap tightly sealed at 25°C.

Cryopreservation

Reagents
Cryoprotective Solution
DMSO, 2.0 mL
Fresh growth medium w/o bacteria, 8.0 mL

Harvest and Preservation

1. Harvest the cells from a culture that is at or near peak density by centrifuging at 650 x g for 5 minutes.
2. If the cell concentration exceeds the required level do not centrifuge, but adjust the concentration to 2 x 10^6 cells/mL with fresh medium. If the concentration is too low, centrifuge at 650 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 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 equal 1 x 10^6 cells/mL and 10% (v/v) DMSO. The time from the mixing of the cell preparation and DMSO stock

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solution to the start of the freezing process 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 add 0.5 mL bacterized ATCC medium 802 to the frozen ampule and place it in a 35°C water bath. Immerse the vial to a level just above the surface of the frozen material. Do not agitate the vial.
9. Immediately after thawing, do not leave in water bath, aseptically remove the contents of the ampule and inoculate onto the surface of a 20 x 100 mm petri plate containing ATCC medium 919 (non-nutrient agar) with an overlay of 15.0 mL bacterized ATCC medium 802.
10. Incubate at 25°C with the cap on loosely.
11. Once the culture is established, transfer 0.5 mL to a T-25 tissue culture flask containing 10.0 mL of ATCC medium 802 bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™).
12. Incubate with the cap tightly sealed at 25°C.

Alternative Thawing Procedure

1. Aseptically add 0.5 mL of sterile exhausted* ATCC medium 802 containing 8% (w/v) sucrose to the ampule. Immediately place in a 35°C water bath, until thawed. Immerse the ampule just sufficient to cover the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically remove the contents of the ampule and gently add the material to the edge of a 20 x 100 mm petri plate containing ATCC Medium 919 (non-nutrient agar) and position on a 15 degree slant. The cell suspension will pool at the edge of the plate.
3. Continue to double the volume of the cell suspension at 10 minute intervals by dropwise addition of exhausted ATCC medium 802 containing 4% sucrose (w/v). When the volume reaches 16.0 mL place the plate in horizontal position and incubate at 25°C.
4. Once the culture has been established subculture into a T-25 flask of bacterized ATCC medium 802 without sucrose.

*Previously-bacterized ATCC medium 802 cleared by growth of bacteria (or by growth of ATCC® PRA-370™ *Tetrahymena* sp., if available), and filter sterilized.

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

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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.