ATCC

Product Sheet

Opisthonecta henneguyi (ATCC® 30600™)

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: *Opisthonecta henneguyi* (ATCC® 30600™)

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: A2
Deposited Name: *Opisthonecta henneguyi* Faure-Fremiet
Depositor: DM Spoon
Isolation: Little Falls runoff area, Maryland shore of Potomac River

Propagation

Growth Conditions
Temperature: 20°C to 25°C
Atmosphere: Aerobic

Medium
ATCC® Medium 802: Sonneborn's Paramecium medium

Instructions for Complete Medium
ATCC Medium 802 may be pre-inoculated with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™) for better growth.

Protocols

Storage and Culture Initiation
This strain comes as dried cysts on shredded filter paper. Dried samples can remain at room temperature for up to one week. If the culture will not be rehydrated within that period, store at 5°C until processed.

1. To rehydrate an ampule, aseptically add 0.5-1.0 mL of sterile ATCC medium 802 or sterile distilled water to the inner shell vial. Aseptically remove the filter paper pellet with a pair of sterile forceps and transfer it 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™).
2. Using a Pasteur pipette, aseptically transfer the remainder of the liquid from the vial to the T-25 flask. Agitate the flask to break apart the filter paper pellet, and incubate the culture at 20-25°C with the cap screwed on tightly. Excystment should occur within a few days.

Culture Maintenance
Subculture at peak density (approximately every 10-14 d) to a fresh T-25 flask of fresh medium in the following manner:

1. Use a cell scraper or rubber policeman to detach adherent cysts and aseptically transfer 0.5 mL to a T-25 tissue culture flask containing 10 mL complete medium.
2. Incubate with the cap tightly sealed at 20-25°C.

Cryopreservation

Reagents
Cryoprotective Solution
DMSO, 2.0 mL
Fresh growth medium, 8.0 mL

Harvest and Preservation

1. Harvest cultures when cells have fully encysted, using a cell scraper or rubber policeman to detach adherent cysts.
2. Adjust the concentration to approximately 2 x 10^5 cysts/mL by centrifugation at 1300 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 be approximately 10^5 cysts/mL and 10.0% (v/v) DMSO. The time from the mixing of the cell preparation and DMSO stock solution to the start of the freezing process should be no less than 15 min and no longer than 60 min.

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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 are stored in either the vapor or liquid phase of a nitrogen freezer.
8. To establish a culture from the frozen state, place the vial 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. Immediately after thawing, do not leave in water bath, aseptically remove the contents of the ampule and inoculate into a T-25 tissue culture flask containing 10 mL ATCC medium 802 bacterized with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™).
9. Incubate with the cap tightly sealed at 20-25°C.
10. Once the culture is established, follow the protocol for maintenance of culture.

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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This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

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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