ATCC

Product Sheet

Micriamoeba tesseris (ATCC® PRA-369™)

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: *Micriamoeba tesseris* (ATCC® PRA-369™)

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: tmp4
Deposited Name: *Micriamoeba tesseris*
Depositor: M Pelandakis
Isolation: Hospital cooling tower, Lyon, France, 2007

Notes

This axenic culture must be fed with heat-killed *Escherichia coli* bacteria (ATCC® 23740™ or similar).

Propagation

Growth Conditions
Temperature: 20°C to 25°C
Atmosphere: Aerobic
Culture System: Axenic

Medium
ATCC® Medium 1034: Modified PYNFH medium (Available from ATCC as ATCC cat. no. 327-X)

Instructions for Complete Medium
Fed with heat-killed *Escherichia coli* bacteria (ATCC® 23740™ or similar)

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 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. 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.
2. Immediately after thawing, aseptically transfer the entire contents to a T-25 flask containing 10 mL complete medium.
3. Add 0.1 to 0.5 mL from a preparation of heat-killed *Escherichia coli* bacteria (ATCC® 23740™ or similar). The quantity of the food-source bacteria to be added will depend on the density of the heat-killed bacterial suspension and the desired rate of growth for *Micriamoeba*, and should be determined empirically.
4. Incubate with the cap tightly sealed at 20-25°C.

Culture Maintenance
Subculture at peak density (approximately every 7-14 d) to a fresh T-25 flask of complete medium in the following manner:

1. Vigorously agitate the flask and aseptically transfer 0.25 mL to a T-25 tissue culture flask containing 10 mL complete medium.
2. Add 0.1 to 0.5 mL from a preparation of heat-killed *Escherichia coli* bacteria (ATCC® 23740™ or similar). The quantity of the food-source bacteria to be added will depend on the density of the heat-killed bacterial suspension and the desired rate of growth for *Micriamoeba*, and should be determined empirically.
3. Incubate with the cap tightly sealed at 20-25°C.

Cryopreservation

Reagents
Cryoprotective Solution
DMSO, 1.5 mL
Fresh complete growth medium, 8.5 mL

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Harvest and Preservation

1. To achieve the best results, set up cultures with several different inocula (i.e., 0.25 mL, 0.5 mL, 1.0 mL). Harvest cultures and pool when the culture that received the lowest inoculum is at or near peak density.
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 10^7cells/mL with fresh growth medium. If the concentration is too low, centrifuge at 1200 to 1600 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 15% (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 between 10^6 and 10^7 cells/mL and 7.5% (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 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 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 35°C water bath (2-3 min). Immerse the vial just sufficiently to cover the frozen material. Do not agitate the vial.
9. Immediately after thawing, aseptically remove the contents of the ampule and inoculate into 10 mL complete medium in a T-25 flask.
10. Add 0.1 to 0.5 mL from a preparation of heat-killed *Escherichia coli* bacteria (ATCC® 23740™ or similar).
11. Incubate with the cap tightly sealed at 20-25°C.
12. 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.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

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.

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Additional information on this culture is available on the ATCC web site at www.atcc.org.