ATCC

Product Sheet

Tetrahymena malaccensis (ATCC® 50066™)

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 malaccensis* (ATCC® 50066™)

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: MP75
Deposited Name: *Tetrahymena malaccensis* Simon et al.
Depositor: EM Simon, E Meyer
Isolation: Jungle stream near campus of University Pertanian Malaysia, Serdang Baru, Malaysia, 1980

Notes

Serum should be omitted from ATCC medium 1034 for cultivation of *Tetrahymena* spp. The serum will not harm the culture over the short term, but it is richer in nutrients than the *Tetrahymena* require, and they may become more dependent upon it over the long term.

Propagation

Growth Conditions
Temperature: 18°C to 25°C
Culture System: Axenic

Medium
ATCC® Medium 1034: Modified PYNFH medium (Available from ATCC as ATCC cat. no. 327-X)
ATCC® Medium 357: Tetrahymena medium
ATCC® Medium 383: Haskins agar for Tetrahymena

Instructions for Complete Medium
Media: ATCC Medium 1034 without serum is used for short-term cultivation. (ATCC medium 1034 is available in a freeze-dried format as cat. no. 327-X; contact ATCC sales for details).

Alternate Media: ATCC Medium 357 can also be used for short-term cultivation. ATCC Medium 383 is used for long-term cultivation.

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 16 x 125 mm screw-capped test tube containing 5 mL of sterile ATCC medium 1034 or 357. Incubate the parent and daughter cultures upright with the caps on loosely at 25°C.

Culture Maintenance
Routine Short-term Maintenance:

1. Inoculate 5.0 mL of ATCC medium 1034 or 357 with 0.1 mL from a *Tetrahymena* culture at or near peak density.
2. Incubate upright at 25°C with cap loosened one half turn.
3. For routine maintenance subculture every 10-14 d.

Routine Long-term Maintenance:

1. Screw the cap on tightly of a *Tetrahymena* culture at or near peak density and invert several times. Aseptically transfer 0.1 mL aliquot to a fresh vessel of ATCC medium 383.
2. Loosen caps one half turn and incubate vertically at 25°C for 1 d.
3. Place cultures at 15°C for 3-6 wk.
4. For routine maintenance subculture every 3-6 wk.

Cryopreservation

Reagents
RM-9 Media for cryopreservation of *Tetrahymena*

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Proteose Peptone (Difco 0120) 5.0 g
Tryptone 5.0 g
K_2HPO_4 0.2 g
Glucose 1.0 g
Liver extract 0.1 g
Glass distilled water 1.0 L

Dissolve components in glass distilled H_2O and autoclave.

Dryl's Salt Solution
0.1 M NaH_2PO_4 . $3H_20$ 10.0 mL
0.1 M Na_2HPO_4 . $7H_20$ 10.0 mL
0.1 M Sodium citrate . $2H_20$ 15.0 mL
0.1 M $CaCl_2$. $2H_20$ 15.0 mL
Distilled water 950.0 mL
Add the first 3 components to the distilled H_2O and mix thoroughly. Add the $CaCl_2$ solution and mix thoroughly. (Adding the solutions in the order indicated will avoid the precipitation of Ca salts.)

Harvest and Preservation

1. Transfer *Tetrahymena* from usual growth medium to RM-9 medium and allow to grow to near peak density.
2. Harvest cells from a culture by centrifugation at 300 x g for 2 min.
3. Adjust concentration of cells to 2 x 10^6/mL in fresh medium.
4. While cells are centrifuging, prepare a 22% (v/v) sterile solution of sterile DMSO in fresh medium.
    a. Add 2.2 mL of DMSO to an ice cold 20 x 150 mm screw-capped test tube;
    b. Place the tube on ice and allow the DMSO to solidify (~5 min) and then add 7.8 mL of ice cold medium;
    c. Invert several times to dissolve the DMSO;
    d. Allow to warm to room temperature.
5. Add a volume of the DMSO solution equal to the cell suspension volume but add in 3 equal aliquots at 2 min intervals. Thus, the final concentration of the preparation will equal 11% (v/v) DMSO and 10^6 cells /mL.
6. Dispense in 0.5 mL aliquots into 1.0 - 2.0 mL sterile plastic screw-capped cryules (special plastic vials for cryopreservation).
7. Place the ampules in a controlled rate freezing unit. The cooling cycle should be initiated no less than 15 min and no longer than 60 min after the addition of the DMSO to the cell preparation. 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 -50°C ampules are plunged into liquid nitrogen.
8. Store in the vapor or liquid phase of a nitrogen refrigerator.
9. To establish a culture from the frozen state aseptically add 0.5 mL sterile Dryl's Salt Solution to an ampule. Immediately place the ampule in a 35°C water bath until thawed (2-3 min). Immerse the ampule just sufficiently to cover the frozen material. Do not agitate the ampule.
10. Immediately after thawing, aseptically remove the contents of the ampule and inoculate into 5.0 mL of fresh medium in a 16 x 125 mm screw-capped test tube with a slightly loosened cap. Incubate at 25°C.

Alternative Thawing Procedure

1. Aseptically add 0.5 mL of sterile modified PYNFH medium (ATCC Medium 1034) 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 adding ATCC medium 1034 containing 4% sucrose (w/v). When the volume reaches 16.0 mL place the plate in horizontal position and incubate at 25°C.
4. On the following day, gently remove the cell suspension for the plate and transfer to a T-25 tissue culture flask. Note the volume of the suspension and add a volume of fresh medium containing 4% sucrose equal to the volume of the cell suspension. Incubate the culture at 25°C.
5. After the culture has been established subculture into fresh medium without sucrose.

References

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

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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.