Tetrahymena americanis Nanney and McCoy

30315™

Description

Strain designation: HAM 3
Deposited As: *Tetrahymena americanis* Nanney and McCoy
Type strain: No
Mating type: IX

Storage Conditions

Product format: Test tube

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Medium:
ATCC Medium 357: Tetrahymena medium
Instructions for complete medium:
ATCC Medium 357 is used for short-term cultivation. (ATCC medium 1034 can also be used for short-term cultivation and is available in a freeze-dried format from ATCC; contact sales for details). ATCC Medium 383 is used for long-term cultivation.
Temperature: 25°C
Culture system: Axenic

Handling Procedures

Reagents for cryopreservation:
RM-9 Media for cryopreservation of *Tetrahymena*

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 $NaH_2PO_4 \cdot 3H_20$	10.0 ml
0.1 M $Na_2HPO_4 \cdot 7H_20$	10.0 ml
0.1 M Sodium citrate $\cdot 2H_20$	15.0 ml
0.1 M $CaCl_2 \cdot 2H_20$	15.0 ml
Distilled water	950.0 ml

Add the first 3 components to the distilled H_2O and mix thoroughly.

Add the $CaC1_2$ solution and mix thoroughly.

(Adding the solutions in the order indicated will avoid the precipitation of Ca salts.)

Cryopreservation: 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 sufficient 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.

CRYOPRESERVATION:

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 culture has been established subculture into fresh

normal medium without sucrose.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Tetrahymena americanis* Nanney and McCoy (ATCC 30315)

References

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

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