



# *Tetrahymena americanis* Nanney and McCoy

205000™

## Description

**Strain designation:** DR 11d

**Deposited As:** *Tetrahymena americanis* Nanney and McCoy

**Type strain:** No

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## Storage Conditions

**Product format:** Test tube

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## 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.

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## 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.

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## Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at [www.atcc.org](http://www.atcc.org).

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## Growth Conditions

### Medium:

ATCC Medium 357: *Tetrahymena* medium

### Instructions for complete medium:

ATCC Medium 357 is used for routine cultivation. (ATCC medium 1034 can also be used for routine cultivation and is available in a freeze-dried format from ATCC; contact sales for details). ATCC Medium 383 can be used to reduce the sub-cultivation in

**Temperature:** 15-27°C

**Culture system:** Axenic

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## Handling Procedures

### Reagents for cryopreservation:

RM-9 Media for cryopreservation of *Tetrahymena*

Proteose Peptone (Difco 0120)	5.0 g
Tryptone	5.0 g
K <sub>2</sub> HPO <sub>4</sub>	0.2 g
Glucose	1.0 g
Liver extract	0.1 g
Glass distilled water	1.0 L

Dissolve components in glass distilled H<sub>2</sub>O and autoclave.

Dryl's Salt Solution

0.1 M NaH <sub>2</sub> PO <sub>4</sub> · 3H <sub>2</sub> O	10.0 ml
0.1 M Na <sub>2</sub> HPO <sub>4</sub> · 7H <sub>2</sub> O	10.0 ml
0.1 M Sodium citrate · 2H <sub>2</sub> O	15.0 ml
0.1 M CaCl <sub>2</sub> · 2H <sub>2</sub> O	15.0 ml
Distilled water	950.0 ml

Add the first 3 components to the distilled H<sub>2</sub>O and mix thoroughly.

Add the CaCl<sub>2</sub> 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<sup>6</sup>/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  $-1^{\circ}\text{C}/\text{min}$  to  $-40^{\circ}\text{C}$ . If freezing unit can compensate for the heat of fusion, maintain rate at  $-1^{\circ}\text{C}/\text{min}$  through heat of fusion. At  $-50^{\circ}\text{C}$  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  $35^{\circ}\text{C}$  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  $25^{\circ}\text{C}$ .

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 frozen 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 a T-25 flask and position the flask on a 15 degree slant. The cell suspension will pool at the bottom of the flask.
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 8.0 ml place the flask in horizontal position with the cap screwed on tightly and incubate at 25°C.
4. On the following day, add 8.0 ml of ATCC medium 1034 (this will dilute the sucrose to 2%). Incubate the culture at 25°C.
5. When the culture has reached peak density, gently agitate and aseptically transfer a 0.1 ml aliquot into 5 ml of ATCC medium 357 in a 16 x 125 mm screw capped glass test tube. Incubate vertically with the cap screwed on loosely.
6. Transfer every 7-10 days by repeating step 5.

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#### Notes

Additional information on this culture is available on the ATCC web site at [www.atcc.org](http://www.atcc.org).

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unintentional spreading of contaminated cultures.

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## 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 205000)

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## References

References and other information relating to this material are available at [www.atcc.org](http://www.atcc.org).

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