Tetrahymena rostrata (Kahl) Corliss

PRA-326™

Description

Strain designation: TRO1
Type strain: No

Storage Conditions

Product format: Test tube
Storage conditions: See handling procedure

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 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).

Alternate Media: ATCC Medium 357 can also be used for sho
Temperature: 18-25°C
Culture system: Axenic

Handling Procedures

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 of Culture:

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 of Culture:

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.

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 . $3H_20$, 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.)

Cryopreservation:

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.

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.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Tetrahymena rostrata* (Kahl) Corliss (ATCC PRA-326)

References

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

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