Pseudotrichomonas keilini Bishop

PRA-328™

Description

Strain designation: NY0170
Deposited As: Pseudotrichomonas keilini
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 2768: PYNFH, MODIFIED In Seawater
Instructions for complete medium: ATCC Medium 2768 is prepared by combining ATCC Media 1525 and 1405 in a 1:4 ratio, supplemented with 5% complete ATCC Medium 1034. Use 13mL per screw-capped test tube to maintain microaerophilic conditions at the tube base.
Grown with mixed bacteria.
Temperature: 20-25°C
Atmosphere: Microaerophilic
Culture system: Xenic

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 13 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 incubate culture at a 15° horizontal slant at 20-25°C for at 2-3 hours before observing the culture. There should be numerous active trophozoites near the base of the tube (some may be attached). If the numbers are low the culture may have been exposed to temperature extremes in transit. Regardless of the state of the culture, the following steps should be taken to ensure the best chance for culture survival:

1. Place the culture tube on ice for 5-10 minutes, then invert 20 times to dislodge any attached cells.
2. Transfer 0.5 and 1.0 mL aliquots to new 16 x 125 mm screw-capped test tubes containing 13 mL complete medium in each.
3. Re-feed the parent culture by centrifuging it at 800-1000 x g for 8-10 min, aspirate most of the supernatant (leaving approximately 1.0-1.5 mL), and resuspend the pellet with fresh growth medium up to 13 mL.
4. Incubate all cultures on a 15° horizontal slant at 20-25°C with the caps on tight.
5. Observe the culture daily and transfer when many trophozoites are observed (typically within 10-14 d).

Culture maintenance:

Subculture approximately every two weeks to fresh tubes of medium in the following manner:

1. Place the culture tube on ice for 5-10 minutes, then invert 20 times to dislodge any attached cells.
2. Transfer 0.5 and 1.0 mL aliquots to new 16 x 125 mm screw-capped test tubes containing 13 mL complete medium in each.
3. Top up the parent culture tube to 13 mL with fresh medium (optionally remove approximately half its volume prior to re-feeding).
4. Incubate the parent and daughter cultures at a 15° horizontal slant at 20-25°C with the caps on tight.

Reagents for cryopreservation: Cryoprotective Solution
DMSO 1.0 mL

Spent culture supernatant 9.0 mL

- **Cryopreservation:**
  1. Harvest cells from multiple culture tubes at or near peak density by placing tubes on ice for 5-10 minutes, inverting several times to dislodge attached cells, followed by centrifugation at 800-1000 x g for 8-10 min.
  2. While cultures are being centrifuged, prepare a 10% (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 previously-reduced culture supernatant. 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.
  3. Adjust the concentration of cells to at least 2 x 10^5/mL in culture supernatant.
  4. Mix the cell preparation and the cryoprotective solution in equal portions. The final concentration of DMSO will be 5%.
  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 vials in a controlled rate freezing unit. 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 -40°C plunge ampules 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. Ampules are stored in either the vapor or liquid phase of a nitrogen refrigerator.
  8. To establish a culture from the frozen state place the vial in a 35°C water bath. Immerse the vial to a level just above the surface of the frozen material. Do not agitate the vial. Immediately after thawing, do not leave in water bath, aseptically remove the contents of the ampule and inoculate into a 16 x 125 mm screw-capped test tube containing 13 mL ATCC Medium 2768. Tightly seal and then invert the culture tube several times to evenly distribute cells.
  9. Incubate the culture on a 15° horizontal slant at 20-25°C with the cap on tight.
  10. Follow the protocol for maintenance of culture.

Notes

This xenic strain may optionally be cultivated with a non-pathogenic strain of *E. aerogenes* or *K. pneumoniae* as a food source. Other unidentified bacterial flora are also present.

Microaerophilic conditions are achieved near the base of the culture tube containing 13 mL medium; it is not necessary to culture inside an anaerobic / microaerophilic chamber with a special atmosphere.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Pseudotrichomonas keilini* Bishop (ATCC PRA-328)

References

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

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