Trichomonas vaginalis Donne

30187™

Description

Strain designation: 165307-1
Deposited As: *Trichomonas vaginalis* Donne
Type strain: No

Storage Conditions

Product format: Frozen
Storage conditions: -80°C or colder for 1 week, vapor phase of liquid nitrogen for long-term storage

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 2

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 2154: LYI Entamoeba medium
ATCC Medium 361: Modified TYM basal medium (ATCC medium 358) with pH adjusted to 6.0 and 0.2-0.5 ml of heat-inactivated horse serum added per tube before use
Instructions for complete medium: Media: ATCC Medium 2154 adjusted to pH 6.0 with the addition of 150 µL sterile 1N HCl per 13 mL of medium (ATCC Medium 2154 is available in a freeze-dried format as cat. no. PRA-2154)

Alternate Media: ATCC Medium 361
Temperature: 35°C
Atmosphere: Anaerobic
Culture system: Axenic

Handling Procedures

Storage and Culture Initiation

Frozen ampules packed in dry ice should either be thawed immediately or stored in liquid nitrogen. If liquid nitrogen storage facilities are not available, frozen ampules may be stored at or below -70°C for approximately one week. **Do not under any circumstance store frozen ampules at refrigerator freezer temperatures (generally -20°C).** Storage of frozen material at this temperature will result in the death of the culture.

1. To thaw a frozen ampule, place it in a 35°C water bath, until thawed (2 to 3 min). Immerse the ampule just sufficient to cover the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically transfer contents to a screw-capped test tube containing either 9 mL of ATCC medium 361 (completed with serum) or 13 mL ATCC Medium 2154 adjusted to pH 6.0. Incubate the tube at 35°C (tube should be vertical for medium 361 or on a 15° horizontal slant for medium 2154).

Culture maintenance:

1. When the culture is at or near peak density, place the tubes on ice for 10 minutes.
2. Gently invert the culture tube 10 times and aseptically transfer a 0.1 mL to 0.4 mL aliquot to a screw-capped test tube containing either 9 mL of ATCC medium 361 (completed with serum) or 13 mL ATCC Medium 2154 adjusted to pH 6.0.
3. Incubate the culture at 35°C (tube should be vertical for medium 361 or on a 15° horizontal slant for medium 2154).
4. Transfer the culture every 3 to 4 days as described in steps 1 through 2. The transfer interval will depend on the quantity of the inoculum and the quality of the medium. This should be empirically determined by examining the culture on a daily basis until the growth cycle has stabilized. Do not allow the culture to overgrow. The culture crashes soon after reaching peak density.

Cryopreservation:

1. Harvest cells from a culture that is at or near peak density by centrifugation at 800 x g for 5 min. The cells grown in a medium containing agar are concentrated by centrifugation, a solid pellet does not form. The soft pellet is resuspended to desired cell concentration with agar-free supernatant.
2. Adjust the concentration of cells to 2 x 10^6 to 2 x 10^7/mL in fresh medium.
3. While cells are centrifuging prepare a 10% (v/v) solution of sterile DMSO in

fresh medium.

a. Add 1.0 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 9.0 mL of ice cold medium.
c. Invert several times to dissolve the DMSO.
d. Allow to warm to room temperature.

4. Mix the cell preparation and the DMSO in equal portions. Thus, the final concentration will be 10^6 to 10^7 cells/mL and 5% (v/v) DMSO. The time from the mixing of the cell preparation and DMSO stock solution before the freezing process is begun should no less than 15 min and no longer than 30 min.
5. Dispense in 0.5 mL aliquots into 1.0 mL to 2.0 mL sterile plastic screw-capped cryules (special plastic vials for cryopreservation).
6. Place the vials in a controlled rate freezing unit. From room temperature cool at -1°C/min to -40°C. If the freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through the heat of fusion. At -40°C plunge 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. The frozen preparations should be stored in either the vapor or liquid phase of a nitrogen refrigerator. Frozen preparations stored below -130°C are stabile indefinitely. Those stored at temperatures above -130°C are progressively less stabile as the storage temperature is elevated. Vials should not be stored above -55°C.
8. To establish a culture from the frozen state place an ampule in a water bath set at 35°C. Immerse the vial just to a level just above the surface of the frozen material. Do not agitate the vial.
9. Immediately after thawing, do not leave in the water bath, aseptically remove the contents of the ampule and inoculate a 16 x 125 mm screw-capped test tube containing either 9 mL of ATCC medium 361 (completed with serum) or 13 mL ATCC Medium 2154 adjusted to pH 6.0.
10. Incubate the culture at 35°C with the cap screwed on tightly (tube should be vertical for medium 361 or on a 15° horizontal slant for medium 2154).

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Trichomonas vaginalis* Donne (ATCC 30187)

References

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

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