Naegleria sturti Dobson et al.

50356™

Description

Strain designation: SWL NG-277
Deposited As: *Naegleria* sp.
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 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 997: Fresh water ameba medium
Instructions for complete medium: ATCC Medium 997 grown with mixed bacteria
Temperature: 25°C
Culture system: Xenic

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 ampoules 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 (2-3 min). Immerse the ampule enough to cover only the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically transfer contents to a ATCC medium 997 petri dish.
3. Distribute the material evenly over the plate using a spread bar Incubate upright at 25°C.

Culture maintenance:

1. Remove an agar block (~5 mm^2), with trophozoites or cysts, from the edge of an agar plate culture and place it in a test tube containing 1 ml of sterile ATCC medium 1325. Agitate to suspend cells from the agar block. Transfer 0.25 ml of the solution to center of each of two fresh plates and spread evenly with a spread bar.
2. Wrap the entire edge of the plate with parafilm and incubate upright at 25°C.
3. Repeat steps 1-3 at 10-14 d intervals.

Cryopreservation:

1. Allow the cells to encyst. To detach cysts from the plate flush the surface with 5 ml fresh ATCC medium 1323 (Page's Balanced Salt Solution). Rub the surface of the plate with a spread bar to detach adhering amoebae.
2. Transfer the cyst suspension to a sterile centrifuge tube.
3. If the cyst concentration does not exceed 2 x 10^6 cysts/ml adjust the suspension to that concentration. To adjust the concentration, centrifuge at 600 x g for 5 min and resuspend the pellet in the volume of fresh medium required to yield 2 x 10^6.
4. While cells are centrifuging prepare a 15% (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 refrigerated medium. 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.
5. Mix the cell preparation and the DMSO in equal portions. Thus, the final

concentration will be at least 10^6 cysts/ml and 7.5% (v/v) DMSO. The equilibration time (the time between addition of DMSO and the start of the cooling cycle) should be no less than 15 min and no longer than 60 min.

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 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.
8. The frozen preparations are stored in either the vapor or liquid phase of a nitrogen freezer.
9. To establish a culture from the frozen state place an ampule in a water bath set at 35°C (2-3 min). Immerse the vial to a level just above the surface of the frozen material. Do not agitate the vial.
10. Immediately after thawing, aseptically remove the contents of the ampule and distribute to the center of a fresh plate of ATCC medium 997. Distribute the material evenly over the plate using a spread bar. Incubate at 25°C.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Naegleria sturti* Dobson et al. (ATCC 50356)

References

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

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