Naegleria galeacystis De Jonckheere

30294™

Description

Strain designation: A.V. 500
Deposited As: *Naegleria* sp.
Type strain: No

Storage Conditions

Product format: Dried

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 *Enterbacter aerogenes*

- ***Temperature:*** 25°C
- ***Culture system:*** Xenic
- ***Incubation:*** grown with Enterobacter aerogenes ATCC 13048

Handling Procedures

Establishing Cultures from Dried State

This strain is grown with *Enterobacter aerogenes* and then shipped as a dried preparation.

1. To rehydrate an ampule, aseptically add 1 ml of sterile distilled water to the inner shell vial. Aseptically remove the filter paper pellet with a pair of forceps, and place it in the center of a plate of ATCC medium 997.
2. Add the liquid remaining in the vial to the plate, tease apart the filter paper pellet, and distribute evenly over the surface of the plate.
3. Incubate the plate upright at 25°C. Trophozoites should be seen within 2-3 d.

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 1323. 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 galeacystis* De Jonckheere (ATCC 30294)

References

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

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Contact Information

ATCC
10801 University Boulevard
Manassas, VA 20110-2209
USA
US telephone: 800-638-6597
Worldwide telephone: +1-703-365-2700
Email: tech@atcc.org or contact your local distributor