



Naegleria gruberi **Schardinger**

30224™

Product Sheet

Description

Strain designation: NEG-M

Deposited As: *Naegleria gruberi* Schardinger

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 1034: Modified PYNFH medium (Available from ATCC as ATCC cat. no. 327-X)

ATCC Medium 710: Nelson's Culture Medium For Naegleria

ATCC Medium 803: M7 medium

ATCC Medium 902: Schuster's axenic Naegleria medium

Instructions for complete medium: Media: ATCC Medium 1034 (ATCC Medium 1034 is available in a freeze-dried format as cat. no. 327-X).

Alternate Media: ATCC Medium 710; ATCC Medium 803; ATCC Medium 902

Temperature: 25°C

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 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 to 3 min). Immerse the ampule just sufficiently to cover only the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically transfer contents to either a plastic screw-capped tube containing 5 mL ATCC medium 1034, or to a T-25 tissue culture flask containing 10 mL ATCC medium 1034. Incubate at 25°C with the cap screwed on tightly (incubate a test tube on a 15° horizontal slant).

Culture maintenance:

1. Vigorously agitate a culture at or near peak density and aseptically transfer 0.1 mL 0.2 mL to a fresh tube or flask of ATCC medium 1034.
2. Incubate at 25°C with the cap screwed on tightly (incubate a test tube on a 15° horizontal slant).

Cryopreservation:

1. Harvest cells from a culture that is at or near peak density by centrifugation at $600 \times g$ for 5 min. Pool the cell pellets into a single tube.
2. Adjust the concentration of cells to $2.0 \times 10^6/\text{mL}$. If the concentration is too low, centrifuge at $600 \times g$ for 5 minutes and resuspend the cell pellet with a volume of supernatant to yield the desired concentration.
3. Prepare a 15% (v/v) sterile DMSO solution in ATCC medium 1034 as follows:
 - a. Add the required volume of DMSO to a glass screw-capped test tube and place on ice. Allow the DMSO to solidify.
 - b. Add the required volume of refrigerated ATCC medium 1034. Dissolve the DMSO by inverting several times. If the DMSO solution is not prepared on ice, an exothermic reaction will occur that may precipitate certain components of the medium.
4. Mix the cell preparation and the DMSO in equal portions. Thus, the final concentration will be 10^6 and 7.5% (v/v) DMSO. The time from the mixing of

the cell preparation and DMSO stock solution to the start of the freezing process should be no less than 15 min and no longer than 60 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 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.
7. The frozen preparations are stored in either the vapor or liquid phase of a nitrogen refrigerator.
8. To establish a culture from the frozen state place an ampule in a water bath set at 35°C. Immerse the vial just sufficiently to cover only 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 into a fresh tube or flask of ATCC medium 1034.
10. Incubate at 25°C with the cap screwed on tightly (incubate a test tube on a 15° horizontal slant).

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Naegleria gruberi* Schardinger (ATCC 30224)

References

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

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