



Product Sheet

Naegleria gruberi (ATCC® 30133™)

Please read this **FIRST**

Storage Temp.
Frozen Cultures:
-70°C for 1 week;
liquid N₂ vapor
for long term
storage



**Freeze-dried
Cultures:**
2-8°C

Live Cultures:
See Protocols
section for
handling
information



Biosafety Level
1

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: *Naegleria gruberi* (ATCC® 30133™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Strain Designation: EG
Deposited Name: *Naegleria gruberi* Schardinger
Depositor: W Balamuth
Isolation: Soil in a eucalyptus grove, Berkeley, CA, 1959

Notes

This culture contains *Escherichia coli*, used as the bacterial food source at time of deposit. Good growth of both the amoeba and bacterial food source may be possible without addition of other bacteria to the culture, though initial growth of the amoebae may be slower when culture is first established.

Propagation

Growth Conditions
Temperature: 25°C

Medium
ATCC® Medium 997: Fresh water amoeba medium
ATCC® Medium 711: PYB

Instructions for Complete Medium

Media: ATCC medium 997 inoculated with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™).

Alternate media: ATCC medium 711 inoculated with bacteria as above. (Note that ATCC medium 711 is a richer formula than ATCC medium 997 and will produce denser and faster bacterial growth. Excess bacterial growth can inhibit growth of amoebae.)

Protocols

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 ampoules 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 the entire contents to a plate of ATCC medium 997. Distribute the material evenly over the plate using a spread bar.
3. Wrap the entire edge of the plate with parafilm and incubate upright at 25°C. Trophozoites should be seen within 2-3 d.

Culture Maintenance

1. Streak an ATCC medium 997 plate with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™) and incubate at 35°C overnight.
2. Remove an agar block (~5 mm²) with trophozoites from the edge of an agar plate culture and invert the block at the edge of the freshly bacterized plate.
3. Wrap the entire edge of the plate with parafilm and incubate upright at 25°C.
4. Repeat steps 1-3 at 10-14 d intervals.
Note: a monoxenic amoeba culture can be established in this manner using any suitable bacterial food source.

Cryopreservation

Reagents

Cryoprotective Solution

DMSO, 1.5 mL
Page's Balanced Salt Solution (or similar), 8.5 mL



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Harvest and Preservation

1. Mix the components in the order listed. When the medium is added to the DMSO the solution will warm up due to chemical heat.
2. Harvest cells from a culture which is at or near peak density by adding 5 mL ATCC medium 1323 (Page's Balanced Salt Solution) and washing cells into suspension. Rub the surface of the plate with a spread bar to detach adhering trophozoites.
3. Adjust the concentration of cells to at least 2×10^6 /mL in fresh medium.
4. Mix the cell preparation and the cryoprotective solution in equal portions.
5. Dispense in 0.5 mL aliquots into 1.0 - 2.0 mL sterile plastic screw-capped cryovials (special plastic vials for cryopreservation).
6. Place vials in a controlled rate freezing unit. From room temperature cool at $-1^\circ\text{C}/\text{min}$ to -40°C . If freezing unit can compensate for the heat of fusion, maintain rate at $-1^\circ\text{C}/\text{min}$ 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 $-1^\circ\text{C}/\text{min}$.)
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, aseptically transfer the contents of the ampule to the center of a fresh plate of ATCC medium 997. Distribute the material evenly over the plate using a spread bar.
9. Wrap the entire edge of the plate with parafilm and incubate upright at 25°C .
10. Follow the protocol for maintenance of culture.



References

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



Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

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Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

Additional information on this culture is available on the ATCC web site at www.atcc.org.

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