**Description**

**Strain Designation:** Neff  
**Deposited Name:** Acanthamoeba sp.  
**Depositor:** RJ Neff  
**Isolation:** Soil, Pacific Grove, CA, 1957

**Propagation**

**Temperature:** 25°C  
**Culture System:** Axenic

**Medium**  
ATCC® Medium 712: PYG w/ Additives

**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 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 such that the lip of the ampule remains above the water line. Thawing time is approximately 2 to 3 minutes. Do not agitate the ampule. Do not leave ampule in water bath after thawed.
2. Immediately after thawing, aseptically transfer contents to a T-25 tissue culture flask or 16 x 125 mm plastic test tube containing 5 mL ATCC Medium 712.
3. Screw the cap on tightly and incubate the tube or flask at 25°C.

**Culture Maintenance**

1. When the culture is at or near peak density, vigorously agitate the culture.
2. Transfer approximately 0.25 ml to a fresh tube or flask containing 5 ml of fresh ATCC medium 712.
3. Screw the caps on tightly and incubate at 25°C (incubate test tubes at a 15° horizontal slant).
4. The amoebae will form an almost continuous sheet of cells on the bottom surface of the flask or test tube. Repeat steps 1-3 at 5-7 d intervals.

**Cryopreservation**

**Harvest and Preservation**

1. To achieve the best results, set up cultures with several different inocula (e.g. 0.25 mL, 0.5 mL, 1.0 mL). Harvest cultures and pool when the culture that received the lowest inoculum is at or near peak density.
2. If the cell concentration exceeds the required level, do not centrifuge, but adjust the concentration to between 2 x 10⁶ and 2 x 10⁷ cysts/mL with fresh medium. If the concentration is too low, centrifuge at 600 x g for 5 min and resuspend the pellet in the volume of fresh medium required to yield the desired concentration.
3. 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.
4. Mix the cell preparation and the DMSO in equal portions. Thus, the final concentration will be between 10² and 10⁷ cells/mL and 7.5% (v/v) DMSO. The time from the mixing of the cell preparation and DMSO stock solution before the freezing process is begun should be no less than 15 min and no longer than 60 min.
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 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

---

**Product Sheet**  
*Acanthamoeba castellanii* (ATCC® 30010™)

**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
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 are stored in either the vapor or liquid phase of a nitrogen freezer.

8. 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 just sufficiently to cover the frozen material. Do not agitate the vial.

9. Immediately after thawing, aseptically remove the contents of the ampule and inoculate into 5 mL of fresh ATCC medium 712 in a T-25 tissue culture flask or plastic 16 x 125 mm screw-capped test tube. Incubate at 25°C.

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

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.

ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

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

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

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.

© ATCC 2018. All rights reserved. ATCC is a registered trademark of the American Type Culture Collection. [08/17]