



# *Acanthamoeba castellanii* (Douglas) Page

50373™

## Description

**Strain designation:** OS4-7B/Neff

**Deposited As:** *Acanthamoeba castellanii* (Douglas) Page

**Type strain:** No

---

## Storage Conditions

**Product format:** Freeze-dried

**Storage conditions:** 2°C to 8°C

---

## 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 submerged 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 submerged 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](http://www.atcc.org).

---

## **Growth Conditions**

**Medium:**

ATCC Medium 712: PYG w/ Additives

**Instructions for complete medium:** ATCC Medium 712

**Temperature:** 25°C

**Culture system:** Axenic

---

## **Handling Procedures**

### **Storage and Culture Initiation**

Freeze dried samples can remain at room temperature for several days. If the cultures will not be rehydrated within that period, store at 5°C until rehydrated.

1. To rehydrate an ampule, add 0.5 ml ice cold ATCC medium 712, containing 12% sucrose, to the freeze dried inner shell vial.
2. Once completely rehydrated, aseptically distribute the rehydrated contents to a plastic 16 X 125 mm screw-capped test tube or a T-25 tissue culture flask containing 5 ml of fresh ATCC medium 712.
3. Incubate the test tube or flask with the caps on tight at 25°C (incubate test tubes horizontally). Trophozoites should be evident within 2-3 d.

**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 10-14 d intervals.

**Cryopreservation:**

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  $2 \times 10^6$  and  $2 \times 10^7$  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  $10^6$  and  $10^7$  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 30 min.
5. Dispense in 0.5 ml aliquots into 1.0 - 2.0 ml sterile plastic screw-capped cryules

- (special plastic vials for cryopreservation).
6. Place the vials in a controlled rate freezing unit. From room temperature cool at  $-1^{\circ}\text{C}/\text{min}$  to  $-40^{\circ}\text{C}$ . If the freezing unit can compensate for the heat of fusion, maintain rate at  $-1^{\circ}\text{C}/\text{min}$  through the heat of fusion. At  $-40^{\circ}\text{C}$  plunge into liquid nitrogen. Alternatively, place the vials in a Nalgene  $1^{\circ}\text{C}$  freezing apparatus. Place the apparatus at  $-80^{\circ}\text{C}$  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. 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  $35^{\circ}\text{C}$  (2-3 min). Immerse the vial just sufficient 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  $25^{\circ}\text{C}$ .
- 

## Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Acanthamoeba castellanii* (Douglas) Page (ATCC 50373)

---

## References

References and other information relating to this material are available at [www.atcc.org](http://www.atcc.org).

---

## Warranty

The product is provided 'AS IS' and the viability of ATCC® products is warranted for 30 days from the date of shipment, provided that the customer has stored and handled the product according to the information included on the product information sheet, website, and Certificate of Analysis. For living cultures, ATCC lists the media

formulation and reagents that have been found to be effective for the product. While other unspecified media and reagents may also produce satisfactory results, a change in the ATCC and/or depositor-recommended protocols may affect the recovery, growth, and/or function of the product. If an alternative medium formulation or reagent is used, the ATCC warranty for viability is no longer valid. Except as expressly set forth herein, no other warranties of any kind are provided, express or implied, including, but not limited to, any implied warranties of merchantability, fitness for a particular purpose, manufacture according to cGMP standards, typicality, safety, accuracy, and/or noninfringement.

---

## Disclaimers

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. Any proposed commercial use is prohibited without a license from ATCC.

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 or complete and the customer bears the sole responsibility of confirming the accuracy and completeness of any such information.

This product is sent on the condition that the customer is responsible for and assumes all risk and responsibility in connection with the receipt, handling, storage, disposal, and use of the ATCC product including without limitation taking all appropriate safety and handling precautions to minimize health or environmental risk. As a condition of receiving the material, the customer agrees that any activity undertaken with the ATCC product and any progeny or modifications will be conducted in compliance with all applicable laws, regulations, and guidelines. This product is provided 'AS IS' with no representations or warranties whatsoever except as expressly set forth herein and in no event shall ATCC, its parents, subsidiaries, directors, officers, agents, employees, assigns, successors, and affiliates be liable for indirect, special, incidental, or consequential damages of any kind in connection with

or arising out of the customer's use of the product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of such materials.

Please see the material transfer agreement (MTA) for further details regarding the use of this product. The MTA is available at [www.atcc.org](http://www.atcc.org).

---

## Copyright and Trademark Information

© ATCC 2023. All rights reserved.

ATCC is a registered trademark of the American Type Culture Collection.

---

## Revision

This information on this document was last updated on 2021-05-19

---

## 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](mailto:tech@atcc.org) or contact your local distributor

---