# *Acanthamoeba byersi* Qvarnstrom et al.

**PRA-411™**

## Description

**Strain designation:** CDC:V621
**Deposited As:** *Acanthamoeba byersi*
**Genotype:** T18

## 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 2

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 712: PYG w/ Additives
**Instructions for complete medium: Media:** ATCC Medium 712 supplemented with 10% heat-inactivated fetal bovine serum (HIFBS)
**Temperature:** 35°C
**Atmosphere:** Ambient

## 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 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 containing 5 mL ATCC Medium 712 supplemented with 10% HIFBS.
3. Screw the cap on tightly and incubate the flask at 35°C.

**Culture maintenance:**

1. When the culture is at or near peak density, scrape the surface of the flask with a cell scraper to detach adhering trophozoites.
2. Transfer approximately 0.25 mL to a fresh flask containing 5 ml of fresh ATCC medium 712 supplemented with 10% HIFBS.
3. Screw the caps on tightly and incubate at 35°C.
4. The amoebae will form an almost continuous sheet of cells on the bottom surface of the flask. Repeat steps 1-3 at 7-10 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 x $10^6$ and 2 x $10^7$cells/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. 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 cryovials.
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 fusion. 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 supplemented with 10% HIFBS in a T-25 tissue culture flask. Incubate at 35°C.

## Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Acanthamoeba byersi* Qvarnstrom et al. (ATCC PRA-411)

## References

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

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## Revision

This information on this document was last updated on 2025-10-14

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