



Balamuthia mandrillaris **Visvesvara et al.**

PRA-291™

Product Sheet

Description

Strain designation: CDC:V451

Type strain: Yes

Storage Conditions

Product format: Frozen

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

Host: Vero (ATCC CCL-81)

Medium:

ATCC Medium 2222: Cell Cultivation Medium for Parasites

Temperature: 35-37°C

Handling Procedures

Cryopreservation:

1. To harvest the *Balamuthia* culture, detach cysts by scraping the inside bottom surface of the flask with a sterile cell scraper.
2. Transfer the cyst suspension to 15 ml plastic centrifuge tubes. Centrifuge at approximately 800 x g for 5 min.
3. Remove all but 0.5 ml of the supernatant from each tube, resuspend the cyst

pellets, and pool them to a single tube.

4. Adjust the concentration of cysts to $2.0 - 4.0 \times 10^5$ cysts/ml with fresh medium or PBS.

NOTE: If the concentration of cysts is too low, centrifuge at $800 \times g$ for 5 min and resuspend in the volume of fresh medium or PBS required to yield the desired concentration.

5. Prepare a cryoprotective solution containing 15% (v/v) DMSO and 6% (v/v) HIFBS in fresh medium or PBS.

6. Mix the cyst preparation and cryoprotective solution in equal portions. The final concentration will be $1.0 - 2.0 \times 10^5$ cysts/ml, 7.5% DMSO, and 3% HIFBS. The time from the mixing of the cyst preparation and cryoprotective solution to the start of the freezing process should be no less than 15 min. and no more than 30 min.

7. Dispense in 0.5 ml aliquots to 1.0-2.0 ml sterile plastic screw-capped cryules (special plastic vials for cryopreservation).

8. 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 $-1^\circ\text{C}/\text{min}$.)

9. Store frozen ampules in either the vapor or liquid phase of a nitrogen refrigerator.

10. 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.

11. Immediately after thawing, aseptically transfer contents to a T-25 tissue culture flask containing a fresh monolayer of ATCC CCL-81 cells and 10 ml ATCC 30-2003 with 3% (v/v) HIFBS.

12. Outgas the flask for 10 seconds with a 95% air, 5% CO_2 gas mixture.

13. Incubate in a 35-37°C CO₂ incubator with the cap screwed on tightly.
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Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Balamuthia mandrillaris* Visvesvara et al. (ATCC PRA-291)

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 2025-10-13

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