



# ***Balamuthia mandrillaris*** **Visvesvara et al.**

**PRA-290™**

## **Description**

**Strain designation:** CDC:V416

**Type strain:** No

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## **Storage Conditions**

**Product format:** Frozen

**Storage conditions:** -80°C or colder for 1 week, vapor phase of liquid nitrogen for long-term storage

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## **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.

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## **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.

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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.

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**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).

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**Growth Conditions****Host:** Vero (ATCC CCL-81)**Medium:**

ATCC Medium 2222: Cell Cultivation Medium for Parasites

**Instructions for complete medium:**

Eagle's Minimum Essential Medium (EMEM) (ATCC cat. 30-2003) with 2 mM L-glutamine and Earle's BSS adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, and 1.0 mM sodium pyruvate; supplemented with 3% fetal bovine serum (ATCC cat. 30-2020). (Contact ATCC Sales to order)

**Temperature:** 35-37°C**Handling Procedures**

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**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  $-70^{\circ}\text{C}$  for approximately one week. **Do not under any circumstance store frozen ampules at refrigerator freezer temperatures (generally  $-20^{\circ}\text{C}$ ).** 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  $35^{\circ}\text{C}$  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 it is thawed.
2. 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.
3. Outgas the flask for 10 seconds with a 95% air, 5%  $\text{CO}_2$  gas mixture.
4. Incubate in a  $35\text{-}37^{\circ}\text{C}$   $\text{CO}_2$  incubator with the cap screwed on tightly.

**Cell Line Maintenance:**

1. To establish a cell 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 sufficiently to cover the frozen material. Do not agitate the vial.
2. Immediately after thawing, aseptically remove the contents of the ampule and inoculate into 10.0 ml of fresh ATCC 30-2003 with 10% (v/v) Heat-Inactivated Fetal Bovine Serum (HIFBS)\* in a T-25 tissue culture flask.
3. Outgas the flask for 10 seconds with a 95% air, 5%  $\text{CO}_2$  gas mixture.
4. Incubate in a  $35\text{-}37^{\circ}\text{C}$   $\text{CO}_2$  incubator with the cap screwed on tightly.
5. Change the medium 1-2 times per week.

\* Fetal bovine serum is available from ATCC (catalog number 30-2020; contact ATCC Sales to order). Serum is heat-inactivated by exposure to  $56^{\circ}\text{C}$  for 30 minutes. This treatment will inactivate proteins of the complement pathway. Remove the serum from the refrigerator and aseptically distribute in 100 ml aliquots to sterile 125 ml screw-capped bottles. Immerse bottles in a  $35^{\circ}\text{C}$  water bath for 5 minutes. Do not directly transfer bottles from the refrigerator to  $56^{\circ}\text{C}$ . Transfer the bottles to a  $56^{\circ}\text{C}$  water bath and begin

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timing for 30 minutes. To avoid contamination, do not allow the level of the water in the bath to come in contact with the lip of the screw cap. It is best to leave one inch between the serum level in the bottle and the lip of the cap and to fill the water bath to a level just slightly above the level of the serum. To assure even heating of the serum, swirl the bottle(s) every ten minutes. Note: Some suppliers provide serum already heat-inactivated.

**Transferring the Cell Line:**

1. When the cell line forms a confluent layer, remove all the medium and replace it with 3 ml of Phosphate Buffered Saline (PBS) (ATCC cat. 30-2200). Incubate T-25 flask at 35-37°C for 25-30 min.
2. Remove all the PBS and replace it with 2 ml of 0.25% (w/v) trypsin dissolved in Hank's Balanced Salt Solution (ATCC cat. 30-2101).
3. Gently distribute the trypsin over the monolayer, remove the trypsin, and place the flask at 35-37°C for 10 min.
4. Add 2 ml of ATCC 30-2003 with 10% (v/v) HIFBS and detach any cells still adherent by alternately aspirating the medium into a pipette and discharging the contents over the monolayer.
5. Distribute the cell suspension in 0.5 ml aliquots to 4 T-25 flasks containing 10 ml fresh ATCC 30-2003 with 10% (v/v) HIFBS.
6. Outgas the flask for 10 seconds with a 95% air, 5% CO<sub>2</sub> gas mixture.
7. Incubate in a 35-37°C CO<sub>2</sub> incubator with the cap screwed on tightly.

**Culture maintenance:**

1. Allow the *Balamuthia* to completely destroy the host cell monolayer and encyst.
2. Remove the medium from a fresh confluent monolayer of CCL-81 cells in a T-25 tissue culture flask and replace it with 10 ml of ATCC 30-2003 with 3% (v/v) HIFBS.
3. To transfer the *Balamuthia* culture, use a sterile cell scraper to detach cysts from the *Balamuthia* culture, agitate gently to evenly suspend the cysts, and aseptically transfer a 0.5 ml aliquot to the flask of CCL-81 cells prepared in step 2.
4. Outgas the flask for 10 seconds with a 95% air, 5% CO<sub>2</sub> gas mixture.
5. Incubate in a 35-37°C CO<sub>2</sub> incubator with the cap screwed on tightly.

**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 x 10<sup>5</sup> cysts/ml with fresh medium or PBS.  
NOTE: If the concentration of cysts is too low, centrifuge at 800 x 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 x 10<sup>5</sup> 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°C/min to -40°C. If freezing unit can compensate for the heat of fusion, maintain rate at -1 C/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°C/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<sub>2</sub> gas mixture.
13. Incubate in a 35-37°C CO<sub>2</sub> incubator with the cap screwed on tightly.

## 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-290)

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## References

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

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## Revision

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