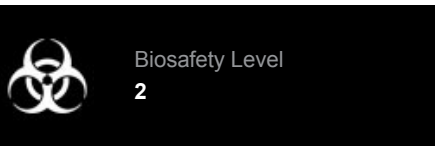
ATCC

Product Sheet

Balamuthia mandrillaris (ATCC® 50209™)

Please read this FIRST



Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: *Balamuthia mandrillaris* (ATCC® 50209™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Strain Designation: CDC:V039
Deposited Name: *Balamuthia mandrillaris* Visvesvara et al.
Depositor: GS Visvesvara
Isolation:
brain tissue from 3 year, 10 month old female mandrill, Papio sphinx, that died of amebic meningoencephalitis, San Diego Zoo, 1986

Propagation

Growth Conditions
Temperature: 35.0°C
Protocol: ATCCNO: 50209 SPEC: Upon arrival place frozen ampule directly into a 35C water bath, transfer thawed contents to 10 ml of fresh medium in a T-25 tissue culture flask containing a monolayer of African green monkey kidney cells (ATCC CRL-1586). The amoebae will completely destroy the monolayer and then encyst. Vigorously agitate the encysted culture and aseptically transfer 0.1 ml to a fresh monolayer of ATCC CRL-1586.

Medium
ATCC® Medium 1156: RP medium

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 10^5 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 10^5 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°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 cells (ATCC CRL-1586 or CCL-81) 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 CO_2 incubator with the cap screwed on tightly.

References

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

Biosafety Level: 2

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.

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2

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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.

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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.

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