# *Balamuthia mandrillaris*, Strain CDC:V188

## Catalog No. NR-46452

## For research use only. Not for human use.

### Contributor:

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### Manufacturer:

BEI Resources

### Product Description:

Protozoa Classification: *Centramoebida*, *Balamuthia*
Species: *Balamuthia mandrillaris*
Strain: CDC:V188
Original Source: *Balamuthia mandrillaris (B. mandrillaris)*, strain CDC:V188 was isolated in 1996 from the brain of a 59-year-old male in Georgia following a traumatic leg amputation and skin abscess and was originally identified as a leptomyxid amoeba.[1-3]
Comment: The mitochondrion genome sequence of *B. mandrillaris*, strain CDC:V188 is available (GenBank: KT175738).

*B. mandrillaris* is a free-living pathogenic amoeba that has been isolated from soil and airborne dust particles throughout the world.[2-4] It is known to cause the fatal infection granulomatous amoebic encephalitis (GAE) in both healthy and immunocompromised humans, as well as in animals.[2,3] *B. mandrillaris* consists of two morphological life cycles, an infective, feeding trophozoite and a dormant double-walled cyst. Infection occurs when the amoeba enters the host through skin wounds or the respiratory system, where it spreads to the central nervous system through the circulatory system. Transmission of infection has also occurred through solid organ transplantation.[4]

Analysis of isolates from various hosts and locations has demonstrated a limited sequence variation in the 18S ribosomal RNA (rRNA) gene and mitochondrial 16S rRNA, and Western blot analysis has demonstrated similarities in antigen patterns of proteins, suggesting that *Balamuthia* infections appear to be from a single, widely-distributed lineage of amoeba.[4,5] Highly-specific PCR methods for identification of isolates based on the mitochondrial small-subunit rRNA gene *rns* have been developed.[3]

### Material Provided:

Each vial of NR-46452 contains approximately 0.5 mL of culture in cryopreservative. Please see Appendix I for cryopreservation instructions.

### Packaging/Storage:

NR-46452 was packaged aseptically in screw-capped plastic cryovials and is provided frozen on dry ice. The product should be stored at cryogenic temperature (-130°C or colder), preferably in the vapor phase of a liquid nitrogen freezer. If liquid nitrogen storage facilities are not available, frozen cryovials may be stored at -70°C or colder for approximately one week.

Note: Do not under any circumstances store vials at temperatures warmer than -70°C. Storage under these conditions will result in the death of the culture. To insure the highest level of viability, the culture should be initiated immediately upon receipt. Any warming of the product during shipping and transfer must be avoided, as this will adversely affect the viability of the product. For transfer between freezers and for shipping, the product may be placed on dry ice for brief periods, although use of a portable liquid nitrogen carrier is preferred. Please read the following recommendations prior to using this material.

### Growth Conditions:

Eagle's Minimum Essential Medium (EMEM) (ATCC® 30-2003™) adjusted to contain 10% (v/v) heat-inactivated fetal bovine serum (HIFBS)
Vero monkey kidney epithelial cells (ATCC® CCL-81™)
Incubation:
Temperature: 37°C
Atmosphere: Aerobic with 5% $CO_2$
Propagation:

1. Place the frozen vial in a 35°C to 37°C water bath and thaw for approximately 2 to 3 minutes. Do not agitate the vial. Do not leave the vial in the water bath after it is thawed.
3. Immediately after thawing, aseptically transfer the contents to a tissue culture flask containing a fresh monolayer of Vero cells (ATCC® CCL-81™) and 10 mL of growth medium containing 10% (v/v) HIFBS.
3. Outgas the flask for 10 seconds with a 95% air, 5% $CO_2$ gas mixture.
4. Incubate in a 37°C $CO_2$ incubator. Observe the culture daily under an inverted microscope for the presence of trophozoites and cysts.

Maintenance:

1. Remove the medium from a fresh confluent monolayer of human foreskin fibroblast cells in a tissue culture flask and replace it with 10 mL medium containing 10% (v/v) HIFBS.
2. Remove the medium from the *Balamuthia* culture and transfer to a 15 mL centrifuge tube. Centrifuge the parasites at 1300 x g for 10 minutes.
3. Remove the supernatant and resuspend the cell pellet in a small volume (0.5 mL to 1.0 mL) of growth medium. Transfer the resuspended pellet to the fresh culture flask prepared in step 1 above. Follow steps 3 and 4 in Propagation.

### Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Balamuthia mandrillaris*, Strain CDC:V188, NR-46452."

## Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

## Disclaimers:

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## References:

1. Gordon, S. M., et al. "Culture Isolation of *Acanthamoeba* Species and Leptomyxid Amebas from Patients with Amebic Meningoencephalitis, Including Two Patients with AIDS." Clin. Infect. Dis. 15 (1992): 1024-1030. PubMed: 1457633.
2. Schuster, F. L. and Visvesvara, G. S. "Axenic Growth and Drug Sensitivity Studies of *Balamuthia mandrillaris*, an Agent of Amoebic Meningoencephalitis in Humans and Other Animals." J. Clin. Microbiol. 34 (1996): 385-388. PubMed: 8789020.
3. Booton, G. C., et al. "Genotyping of *Balamuthia mandrillaris* Based on Nuclear 18S and Mitochondrial 16S rRNA Genes." Am. J. Trop. Med. Hyg. 68 (2003): 65-69. PubMed: 12556151.
4. Kucerova, Z., et al. "Identification of Antigenic Targets for Immunodetection of *Balamuthia mandrillaris* Infection." Clin. Vaccine Immunol. 18 (2011): 1297-1301. PubMed: 21653740.
5. Booton, G. C., et al. "Identification of *Balamuthia mandrillaris* by PCR Assay Using the Mitochondrial 16S rRNA Gene as a Target." J. Clin. Microbiol. 41 (2003): 453-455. PubMed: 12517892.
6. Visvesvara, G. S., F. L. Schuster and A. J. Martinez. "*Balamuthia mandrillaris*, N. G., N. Sp., Agent of Amebic Meningoencephalitis in Humans and Other Animals." J. Eukaryot. Microbiol. 40 (1993): 504-514. PubMed: 8330028.
7. Visvesvara, G. S., et al. "Leptomyxid Ameba, a New Agent of Amebic Meningoencephalitis in Humans and Animals." J. Clin. Microbiol. 28 (1990): 2750-2756. PubMed: 2280005.

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## APPENDIX I: CRYOPRESERVATION

1. To harvest *Balamuthia mandrillaris*, remove the media containing trophozoites and cysts from a series of infected cultures in T75 flasks and transfer to 15 mL plastic centrifuge tubes. Centrifuge at 1300 x g for 10 min.
2. Remove all but 0.5 mL of the supernatant from each tube, resuspend the cell pellets, and pool them into a single tube.
3. Adjust the trophozoite concentration to 1.0 to 2.0 x $10^6$ cells/mL using fresh growth medium.
   Note: If the concentration of parasites is too low, centrifuge at 1300 x g for 10 min and resuspend in a smaller volume of fresh medium to yield the desired parasite concentration.
4. Mix equal volumes of parasite suspension and fresh medium containing 15% DMSO to yield a final concentration of 0.5-1.0 x $10^6$ cells/mL in 7.5% DMSO. The freezing process should start 15 to 30 minutes following the addition of cryoprotective solution to the parasite suspension.
   Note: To prevent culture contamination, penicillin-streptomycin solution (ATCC® 30-2300) may be added to a final concentration of 50 to 100 U/mL penicillin and 50 to 100 µg/mL streptomycin.
5. Dispense 0.5 mL aliquots into 1 to 2 mL sterile plastic screw-capped vials for cryopreservation.
6. Place the vials in a controlled rate freezing unit. From room temperature cool the vials 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 this phase. At -40°C, plunge vials into liquid nitrogen. Alternatively, place the vials in a Nalgene 1°C freezing container. Place the container at -80°C for 1.5 to 2 hours and then plunge vials into liquid nitrogen.
7. Store in either the vapor or liquid phase of a nitrogen refrigerator (-130°C or colder).