

Balamuthia mandrillaris, Strain CDC:V433

Catalog No. NR-46454

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Product Description: *Balamuthia mandrillaris* (*B. mandrillaris*), strain CDC:V433 was isolated in 1993 from the brain of a 20-year-old male Thoroughbred horse in California.

Lot¹: 64508698

Manufacturing Date: 21NOV2016

TEST	SPECIFICATIONS	RESULTS
Cellular Morphology²	Report results	Rounded, refractile, non-motile
Genotyping³ Sequencing of 18S ribosomal RNA gene (~ 380 base pairs)	Consistent with <i>B. mandrillaris</i>	Consistent with <i>B. mandrillaris</i>
Functional Activity by PCR Amplification³ 18S ribosomal RNA gene	~ 2200 base pair amplicon	~ 2200 base pair amplicon
Viable Cell Count by Hemocytometry^{2,4}	> 10 ⁵ cells/mL	3.52 × 10 ⁶ cells/mL
Viability^{2,5}	Viable parasites	Viable parasites
Sterility (21-day incubation)² Harpo's HTYE broth ⁶ , 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic DMEM with 10% FBS, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic	No growth No growth No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth No growth No growth
Mycoplasma Contamination² DNA detection by PCR	None detected	None detected

¹NR-46454 was produced by cultivation of the deposited material in Vero cells (ATCC® CCL-81™) with Eagle's Minimum Essential Medium (EMEM; ATCC® 30-2003™) adjusted to contain 10% heat-inactivated fetal bovine serum (HIFBS). After a series of passages with Mycoplasma removal agent, the cell suspension was used to inoculate fresh Vero cells and media. The culture was propagated for 5 days at 37°C in an aerobic atmosphere with 5% CO₂ until lysis of the host cell monolayer was reached. Cells were harvested and suspended in fresh EMEM and 7.5% (final %) DMSO cryopreservative to produce this lot.

²Testing completed on vial, post-freeze material.

³Testing completed on bulk material prior to vialing and freezing.

⁴Only viable trophozoite forms of the parasite were counted.

⁵Viable parasites were observed after 1 day at 37°C in an aerobic atmosphere with 5% CO₂ in Vero cells with EMEM (ATCC® 30-2003™) adjusted to contain 10% HIFBS.

⁶Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Date: 29 JUN 2017

Signature:

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