b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-46455

Balamuthia mandrillaris, Strain CDC:V426

Catalog No. NR-46455

This reagent is the tangible property of the U.S. Government.

Product Description: *Balamuthia mandrillaris* (*B. mandrillaris*), strain CDC:V426 was isolated in 1999 from the brain of a 17-year-old male in California, USA.

Lot[1]: 70000223 **Manufacturing Date: 29DEC2016**

TEST	SPECIFICATIONS	RESULTS
Cellular Morphology[2]	Report results	Trophozoites present
Genotyping[3] Sequencing of 18S ribosomal RNA (rRNA) gene (~ 790 base pairs)	Consistent with *B. mandrillaris*	Consistent with *B. mandrillaris*
Functional Activity by PCR Amplification[3] 18S rRNA gene	~ 2200 base pair amplicon	~ 2200 base pair amplicon
Viable Cell Count by Hemocytometry[3,4]	> 10^5 cells/mL	2 × 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 Contamination[3] DNA detection by PCR	None detected	None detected

[1]NR-46455 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 cells were harvested and used to inoculate fresh Vero cells and media. The culture was propagated for 7 days at 37°C in an aerobic atmosphere with 5% CO_2 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.
[2]Testing completed on vialed, post-freeze material.
[3]Testing completed on bulk material prior to vialing and freezing.
[4]Only viable trophozoite forms of the parasite were counted.
[5]Viable cells and signs of infection were observed after 4 days at 37°C in an aerobic atmosphere with 5% CO_2 in Vero cells with EMEM (ATCC® 30-2003™) adjusted to contain 10% HIFBS.
[6]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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