b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-46453

Balamuthia mandrillaris, Strain CDC:V194

Catalog No. NR-46453

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

Product Description: *Balamuthia mandrillaris* (*B. mandrillaris*), strain CDC:V194 was isolated in 1990 from the brain of a 60-year-old male patient with chronic alcoholism from Nevada, USA, who had seizures and hemiparesis.

Lot[1,2]: 64377586 **Manufacturing Date: 22JUN2016**

TEST	SPECIFICATIONS	RESULTS
Genotyping Sequencing of 18S ribosomal RNA gene (~ 380 base pairs)	Consistent with *B. mandrillaris*	Consistent with *B. mandrillaris*[3]
Functional Activity by PCR Amplification 18S ribosomal RNA gene	Report results	~ 2500 base pair amplicon
Viable Cell Count by Hemocytometry (pre-freeze)[4]	> 10^5 cells/mL	1.35 × 10^6 cells/mL
Viability[5]	Viable parasites	Viable parasites
Sterility (21-day incubation) 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 DNA detection by PCR	None detected	None detected

[1]NR-46453 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). The culture was propagated for 5 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 media and 7.5% (final %) DMSO cryopreservative, to produce this lot.

[2]Quality control testing completed on post-freeze material unless specified as pre-freeze.

[3]Although the sequence analysis identified the organism as *B. mandrillaris*, the results produced a mixed template. This may have resulted from the non-monoclonal isolate (this isolate may consist of more than one genotype of *Balamuthia*) or the fact that *Balamuthia* is a diploid organism with regions of heterozygosity in the 18S rRNA gene.

[4]Only viable trophozoite forms of the parasite were counted.

[5]Viable parasites were observed after 8 days at 37°C in a humidified, 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.

Date: 01 MAR 2017 **Signature:**

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