

***Balamuthia mandrillaris*, Strain CDC:V188**

Catalog No. NR-46452

Product Description: *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.

Lot¹: 62630797

Manufacturing Date: 19JUN2014

TEST	SPECIFICATIONS	RESULTS
Genotyping Sequencing of 18S ribosomal RNA gene (~ 520 base pairs)	Consistent with <i>B. mandrillaris</i>	Consistent with <i>B. mandrillaris</i> ²
Functional Activity by PCR Amplification 18S ribosomal RNA gene	Report results	~ 2500 base pair amplicon
Viable Cell Count by Hemocytometry (pre-freeze)	> 10 ⁵ cells/mL	~ 1 x 10 ⁶ cells/mL
Viability (post-freeze)³	Viable cells	Viable cells
Sterility (21-day incubation) Harpo's HTYE broth ⁴ , 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

¹NR-46452 was produced by cultivation of the deposited material in Vero cells (ATCC[®] CCL-81™) with EMEM (ATCC[®] 30-2003™: adjusted to contain 10% heat-inactivated fetal bovine serum). After 8 passages with Mycoplasma removal agent, 0.5 mL of cell suspension was added to fresh Vero cells and media. The culture was propagated for 4 days at 37°C in 95% air, 5% CO₂, 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.

²Although 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 and regions of heterozygosity in the 18S rRNA gene are expected.

³Viable cells were observed after 1 day under cultivation conditions.

⁴Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Date: 09 NOV 2015

Signature:



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