

Certificate of Analysis for NR-46458

Balamuthia mandrillaris, Strain CDC:V619

Catalog No. NR-46458

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

Product Description: Balamuthia mandrillaris (B. mandrillaris), strain CDC:V619 was isolated in 2010 from the cerebrospinal fluid of a 27-year-old male patient with granulomatous amebic encephalitis following a kidney transplant in Mississippi, USA.

Lot1: 70005223 Manufacturing Date: 28APR2017

| TEST | SPECIFICATIONS | RESULTS |
|--|--|---|
| Cellular Morphology ² | Report results | Trophozoites and cysts present |
| Genotyping ³ Sequencing of 18S ribosomal RNA (rRNA) gene (~ 1970 base pairs) | ≥ 99% sequence identity to B. mandrillaris type strain (GenBank: LFUI01000381.1) | 99.4% sequence identity to <i>B. mandrillaris</i> type strain (GenBank: LFUI01000381.1) |
| 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 per mL | 2.5 × 10 ⁶ cells per 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 |
| Mycoplasma Contamination³ DNA detection by PCR | None detected | None detected |

¹NR-46458 was produced by cultivation of the deposited material in Vero cells (ATCC® CCL-81™) with Eagle's Minimum Essential Medium (EMEM; ATCC® 30-2003™) supplemented with 10% heat-inactivated fetal bovine serum (HIFBS). The culture was propagated for 7 days at 37°C in an aerobic atmosphere with 5% CO₂ until lysis of the host cell monolayer was reached.

/Heather Couch/

Heather Couch 09 OCT 2018

Program Manager or designee, ATCC Federal Solutions

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

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BEI Resources

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²Testing completed on vialed, post-freeze material.

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

⁴Only viable trophozoite forms of the parasite were counted.

⁵Viable cells and signs of infection were observed after 6 days 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.