

Certificate of Analysis for NR-49906

Chikungunya Virus, SV 0442-95

Catalog No. NR-49906

Product Description: Cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells¹ infected with chikungunya virus (CHIKV), SV 0442-95

Passage History: MK1V1/V3 (Prior to deposit at BEI Resources/BEI Resources); MK# = Number of passages in LLC-MK2 cells; V# = Number of passages in Vero cells²

Lot³: 64108461 Manufacturing Date: 09JUN2016

| TEST | SPECIFICATIONS | RESULTS |
|--|---|---|
| Infectivity in Vero E6 Cells | Report results | Cell rounding and detachment |
| Sequencing of Species-Specific Region (939 nucleotides) | Consistent with CHIKV | Consistent with CHIKV ⁴ |
| Titer by TCID ₅₀ Assay ^{5,6} in Vero E6 Cells ¹ | Report results | 1.6 × 10 ⁷ TCID ₅₀ per mL |
| Amplification of CHIKV Sequence by RT-PCR | ~ 1070 bp amplicon | ~ 1070 bp amplicon |
| Sterility (21-day incubation) Harpo's HTYE broth ⁷ , 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C and 5% CO ₂ | No growth | No growth |
| Mycoplasma Contamination Agar and broth culture (14-day incubation at 37°C) DNA detection by PCR of extracted Test Article nucleic acid | None detected None detected | None detected None detected |

¹Vero 76, clone E6; ATCC[®] CRL-1586™

Date: 30 MAR 2017 Signatur

BEI Resources Authentication

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²The second virus passage at BEI Resources was performed by lipofectamine-mediated transfection of extracted viral nucleic acid in order to remove contaminating mycoplasma.

³Grown in Eagle's Minimum Essential Medium containing Earle's Balanced Salt Solution, non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate and 1.5 g/L of sodium bicarbonate (ATCC® 30-2003) supplemented with 2% fetal bovine serum (ATCC® 30-2020) for 2 days at 37°C with 5% CO₂

⁴Sequence information for CHIKV, SV 0442-95 is not available in the NCBI database; nucleotide sequence obtained for NR-49906, Lot No. 64108461 is ~ 99% identical to CHIKV, SV0444-95, which was also isolated from a human in Bangkok, Thailand in 1995.

⁵The Tissue Culture Infectious Dose 50% (TCID₅₀) endpoint is the 50% infectious endpoint in cell culture. The TCID₅₀ is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated, just as a Lethal Dose 50% (LD₅₀) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID₅₀ provides a measure of the titer (or infectivity) of a virus preparation.

⁶4 days at 37°C and 5% CO₂

⁷Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.