

Certificate of Analysis for NR-49741

Chikungunya Virus, LR 2006-OPY1

Catalog No. NR-49741

Product Description: Cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells¹ infected with chikungunya virus (CHIKV), LR 2006-OPY1

Passage History: V5/V4 (Prior to deposit at BEI Resources/BEI Resources); V# = Number of passages in Vero cells²

Lot³: 63802502 Manufacturing Date: 11MAR2016

TEST	SPECIFICATIONS	RESULTS
Infectivity in Vero E6 Cells	Report results	Cell rounding and detachment
Sequencing of Species-Specific Region (897 nucleotides)	Consistent with CHIKV, LR 2006-OPY1	100% identity with CHIKV, LR 2006-OPY1 (GenBank: KT449801)
Titer by TCID ₅₀ Assay ^{4,5} 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 ⁶ , 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: 09 AUG 2016 Signat

BEI Resources Authentication

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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²The second virus passage at BEI Resources was performed by lipofectamine 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 3 days at 37°C with 5% CO₂

⁴The 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.

⁵⁵ days at 37°C and 5% CO2

⁶Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.