

Certificate of Analysis for NR-49901

Chikungunya Virus, H 20235-St. Martin-2013

Catalog No. NR-49901

Product Description: Cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells¹ infected with chikungunya virus (CHIKV), H 20235-St. Martin-2013

Passage History: XV2/V2 (Prior to deposit at BEI Resources/BEI Resources); X = Unknown; V# = Number of passages in Vero cells

Lot²: 63965672 Manufacturing Date: 11MAR2016

TEST	SPECIFICATIONS	RESULTS
Infectivity in Vero E6 Cells ¹	Report results	Cell rounding and detachment
Sequencing of Species-Specific Region (906 nucleotides)	Consistent with CHIKV	Consistent with CHIKV ³
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: 03 AUG 2016

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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²Grown 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

³ Sequence information for CHIKV, H 20235-St. Martin-2013 is not available in the NCBI database; nucleotide sequence obtained for NR-49901, Lot No. 63965672 is ~ 99% identical to numerous CHIKV strains isolated in the Caribbean.

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.

⁵⁴ days at 37°C and 5% CO₂

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