

Certificate of Analysis for NR-13220

Chikungunya Virus, S-27

Catalog No. NR-13220

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

Product Description: Cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells (Vero E6)¹ infected with chikungunya virus, S-27.

Lot²: 61216441 Manufacturing Date: 12OCT2012

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using Vero E6 Cells ¹	Report results	Rounding and detachment
Sequencing of Species-Specific Region (~ 1020 nt)	Consistent with chikungunya virus	100% identity to chikungunya virus (GenBank: AF369024)
Titer by TCID ₅₀ Assay ^{3,4} in Vero E6 Cells ¹	Report results	8.9 × 10 ⁷ TCID ₅₀ per mL
Functional Activity by RT-PCR Assay	~ 1180 bp amplicon	~ 1180 bp amplicon
Sterility (21-day incubation) Harpo's HTYE broth ⁵ , 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 E6 (Vero C1008) cells: ATCC[®] CRL-1586™

Date: 02 APR 2013 Signature: Sign

Title: Technical Manager, BEI Authentication or designee

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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 2 days at 37°C with 5% CO₂.

³The 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.

 $^{^45}$ days at 37°C and 5% CO_2

⁵Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.