b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-49905

Chikungunya Virus, PM 2951

Catalog No. NR-49905

Product Description: Cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells[1] infected with chikungunya virus (CHIKV), PM 2951

Passage History: SM3V2/V2 (Prior to deposit at BEI Resources/BEI Resources); SM# = Number of passages in suckling mice; V# = Number of passages in Vero cells

Lot[2]: 64108460 **Manufacturing Date: 12MAY2016**

TEST	SPECIFICATIONS	RESULTS
Infectivity in Vero E6 Cells	Report results	Cell rounding and detachment
Sequencing of Species-Specific Region (1008 nucleotides)	Consistent with CHIKV, PM 2951	100% identity with CHIKV, PM 2951 (GenBank: HM045785)
Titer by $TCID_{50}$ Assay[3,4] in Vero E6 Cells[1]	Report results	1.6×10^8 $TCID_{50}$ per mL
Amplification of CHIKV Sequence by RT-PCR	~ 1150 bp amplicon	~ 1150 bp amplicon
Sterility (21-day incubation)		
Harpo's HTYE broth[5], 37°C and 26°C, aerobic	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
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)	None detected	None detected
DNA detection by PCR of extracted Test Article nucleic acid	None detected	None detected

[1]Vero 76, clone E6; ATCC® CRL-1586™

[2]Grown 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% CO_2

[3]The Tissue Culture Infectious Dose 50% ($TCID_{50}$) endpoint is the 50% infectious endpoint in cell culture. The $TCID_{50}$ 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% (LD_{50}) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the $TCID_{50}$ provides a measure of the titer (or infectivity) of a virus preparation.

[4]7 days at 37°C and 5% CO_2

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

Date: 05 OCT 2016 **Signature:** Michael R. Gyrla

BEI Resources Authentication

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