SUPPORTING INFECTIOUS DISEASE RESEARCH

Yellow Fever Virus, CAREC M2-09

Catalog No. NR-50062

Product Description: Cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells¹ infected with yellow fever virus (YFV), CAREC M2-09

Passage History: V1/V3 (Prior to deposit at BEI Resources/BEI Resources); V# = Number of passages in Vero cells²

Lot³: 64541073

Manufacturing Date: 17MAY2017

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using Vero Cells ¹	Report results	Cell rounding and sloughing
Sequencing of Species-Specific Region (868 nucleotides)	Consistent with YFV	Consistent with YFV ⁴
Titer by TCID₅₀ Assay ^{5,6} in Vero Cells ¹	Report results	2.8 × 10 ⁷ TCID ₅₀ per mL
Amplification of Yellow Fever Virus Sequence by RT-PCR	~ 1030 bp amplicon	~ 1030 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 No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth 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: ATCC[®] CCL-81[™]

²The second viral 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 5 days at 37°C with 5% CO₂.

⁴Sequence information for the region of viral genome coding for non-structural proteins for YFV, CAREC M2-09 is not available in the NCBI database; nucleotide sequence obtained for NR-50062 lot 64541073 is 100% identical to the closely related YFV strain TVP11767 (see Auguste, A. J., et al. "Yellow Fever Virus Maintenance in Trinidad and Its Dispersal throughout the Americas." J. Virol. 84 (2010): 9967-9977. PubMed: 20631128.) and consistent with numerous YFV strains.

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.

⁶7 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.

Certificate of Analysis for NR-50062

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Date: 14 DEC 2017

Signature: Michael Q. My

BEI Resources Authentication

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