

Yellow Fever Virus, CAREC M2-09, Gamma-Irradiated

Catalog No. NR-50890

Product Description: Cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells¹ infected with Yellow Fever Virus (YFV), CAREC M2-09 was gamma-irradiated (5×10^6 RADs) on dry ice.

Lot²: 70012729

Manufacturing Date: 15MAR2018

TEST ³	SPECIFICATIONS	RESULTS
Pre-Inactivation Identification by Infectivity in Vero cells¹	Cell rounding and detachment	Cell rounding and detachment
Pre-Inactivation Sequencing of Species-Specific Region (~ 920 nucleotides)	Consistent with YFV	Consistent with YFV ⁴
Pre-Inactivation Titer by TCID₅₀ Assay^{5,6} in Vero cells¹ by Cytopathic Effect	Report results	1.6×10^4 TCID ₅₀ per mL
Pre-Inactivation Amplification of YFV Sequence by RT-PCR	~ 1030 base pair amplicon	~ 1030 base pair amplicon
Pre-Inactivation 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 Blood agar, 37°C, aerobic 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
Pre-Inactivation 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
Post-Inactivation Viral Genome Copy Number Droplet Digital RT-PCR ⁸	Report results	1.8×10^6 genome copies per μ L
Post-Inactivation Virus Inactivation Cell culture safety test for residual virus ⁹ NR-50890 was inoculated on Vero cells ¹ and evaluated for cytopathic effect, viral antigen expression by indirect immunofluorescence assay ¹⁰ , and presence of viral RNA by real-time RT-PCR after serial passage ¹¹	No recovered virus No viable virus detected	No recovered virus No viable virus detected

¹*Cercopithecus aethiops* kidney epithelial cells (Vero; ATCC® CCL-81™)

²Source of irradiated antigen: BEI Resources NR-50062 lot 70010132

³All tests were completed pre-inactivation, unless otherwise specified.

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 70010132 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.

⁵Grown 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 7 days at 37°C with 5% CO₂

⁶The 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.

⁷Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

⁸ddPCR data was obtained post-vial from 6 replicates on the BioRad QX200 Droplet Digital PCR (ddPCR™) System

⁹Performed at University of Texas Medical Branch, Galveston, Texas, USA

¹⁰Using Monoclonal Anti-Flavivirus Group Antigen, Clone D1-4G2-4-15 (BEI Resources NR-50327)

¹¹The inactivated virus preparation was plated on Vero cells and incubated for 14 days at 37°C and 5% CO₂; cell lysate and supernatant from these cultures were passaged to fresh monolayers of Vero cells and incubated for 14 days at 37°C and 5% CO₂.

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