

Yellow Fever Virus, INHRR 7a-05

Catalog No. NR-50071

Product Description: Cell lysate and supernatant from *Aedes albopictus* mosquito larval epithelial cells¹ infected with yellow fever virus (YFV), INHRR 7a-05

Passage History: XC1/C3 (Prior to deposit at BEI Resources/BEI Resources); X = Unknown; C# = Number of passages in C6/36 cells²

Lot³: 64541080

Manufacturing Date: 02JUN2017

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using C6/36 Cells ¹	Report results	Cell rounding and detachment
Identification by Indirect Fluorescent Antibody (IFA) Assay ⁴	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (911 nucleotides)	Consistent with YFV	Consistent with YFV ⁵
Titer by TCID ₅₀ Assay ^{6,7} in C6/36 Cells ¹ with IFA Readout ⁴	Report results	2.8 × 10 ⁸ 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 ⁸ , 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 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

¹*Aedes albopictus* clone C6/36 cells (ATCC® CRL-1660™)

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 7 days at 28°C with 5% CO₂.

⁴Using Mouse Anti-Yellow Fever Virus Antibody, clone 2D12 (Bio-Rad 9801-8006)

⁵Complete sequence information for YFV, INHRR 7a-05 is not available in the NCBI database; nucleotide sequence obtained for NR-50071 lot 64541080 is 99% identical to the closely related YFV strain 6A (Auguste, A. J., et al. "Enzootic Transmission of Yellow Fever Virus, Venezuela." *Emerg. Infect. Dis.* 21 (2015): 99-102. PubMed: 25531105) and consistent with numerous YFV strains.

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.

⁷7 days at 28°C and 5% CO₂

⁸Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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