

**Yellow Fever Virus, INHRR 10a-10**

**Catalog No. NR-50063**

**Product Description:** Cell lysate and supernatant from *Aedes albopictus* mosquito larval epithelial cells<sup>1</sup> infected with yellow fever virus (YFV), INHRR 10a-10

**Passage History:** XC1/C3 (Prior to deposit at BEI Resources/BEI Resources); X = Unknown; C# = Number of passages in C6/36 cells<sup>2</sup>

**Lot<sup>3</sup>: 64541078**

**Manufacturing Date: 19JUN2017**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using C6/36 Cells <sup>1</sup>	Report results	Cell rounding and detachment
Identification by Indirect Fluorescent Antibody (IFA) Assay <sup>4</sup>	Fluorescence observed	Fluorescence observed
Sequencing of Species-Specific Region (911 nucleotides)	Consistent with YFV	Consistent with YFV <sup>5</sup>
Titer by TCID <sub>50</sub> Assay <sup>6,7</sup> in C6/36 Cells <sup>1</sup> with IFA Readout <sup>4</sup>	Report results	8.9 × 10 <sup>7</sup> TCID <sub>50</sub> per mL
Amplification of Yellow Fever Virus Sequence by RT-PCR	~ 1030 bp amplicon	~ 1030 bp amplicon
<b>Sterility (21-day incubation)</b> Harpo's HTYE broth <sup>8</sup> , 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 <sub>2</sub>	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
<b>Mycoplasma Contamination</b> 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

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

<sup>2</sup>The second viral passage at BEI Resources was performed by lipofectamine-mediated transfection of extracted viral nucleic acid in order to remove contaminating mycoplasma.

<sup>3</sup>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 4 days at 28°C with 5% CO<sub>2</sub>.

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

<sup>5</sup>Complete sequence information for YFV, INHRR 10a-10 is not available in the NCBI database; nucleotide sequence obtained for NR-50063 lot 64541078 is 100% identical to the closely related YFV strain 10A (GenBank: KM388816; 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.

<sup>6</sup>The Tissue Culture Infectious Dose 50% (TCID<sub>50</sub>) endpoint is the 50% infectious endpoint in cell culture. The TCID<sub>50</sub> 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<sub>50</sub>) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID<sub>50</sub> provides a measure of the titer (or infectivity) of a virus preparation.

<sup>7</sup>7 days at 28°C and 5% CO<sub>2</sub>

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

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