

Certificate of Analysis for NR-116

Yellow Fever Virus, 17D

Catalog No. NR-116

Product Description: Yellow Fever Virus (YFV), 17D was derived from the virulent Asibi strain of YFV by in vitro passage in chicken embryo tissue. The Asibi strain was isolated in 1927 by inoculating rhesus macagues with the blood of a West African patient.

Passage History: Parent Strain (Asibi): MK53/MEmb(mince)18/CEmb(whole embryo mince)50; Subline Strain (17D) (Prior to deposit at BEI Resources): CEmb(less CNS)152/CE3/CEmb(less CNS)1/CE8/V2; Subline Strain (17D) (BEI Resources): V2; MK = Monkey Kidney; MEmb = Mouse embryo; CEmb = Chicken embryo; CEmb(less CNS) = Chicken embryo (central nervous system removed); CE = Embryonated chicken eggs; V = Vero cells¹

Lot²: 70018947 Manufacturing Date: 18OCT2018

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero¹ cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (~ 870 nucleotides)	≥ 98% identity with YFV, 17D (GenBank: X03700.1)	99.9% identity with YFV, 17D (GenBank: X03700.1)
Titer by TCID₅₀ Assay³,⁴ in Vero cells¹ by Cytopathic Effect	Report results	2.8 × 10 ⁸ TCID ₅₀ per mL
Amplification of YFV Sequence by RT-PCR	~ 1000 base pair amplicon	~ 1000 base pair amplicon
Sterility (21-day incubation)		
Harpo's HTYE broth⁵, 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
Blood agar, 37°C, aerobic	No growth	No growth
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

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

/Heather Couch/

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Program Manager or designee, ATCC Federal Solutions

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²Lot 70018947 of NR-116 was produced by infecting Vero cells with BEI Resources NRS-116 lot 7496111 and incubating 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.

³The 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

⁴Assay plates were incubated 7 days at 37°C and 5% CO₂.

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