

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-115

Yellow Fever Virus (YFV), 17D

Catalog No. NR-115

Derived from ATCC® VR-1268

Product Description: Cell lysate and supernatant from African green monkey kidney (Vero) cells¹ infected with YFV, 17D.

Lot²: 7496108 Manufacturing Date: 03OCT2006

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero Cells ¹	Report results	Cell rounding and cell lysis
Identification by Indirect Fluorescent Antibody Assay ³	Fluorescence observed	Fluorescence observed
Sequencing of YFV Specific Sequence (~ 275 bp)	Identical to GenBank X03700 (YFV, 17D vaccine strain)	Identical to GenBank X03700 (YFV, 17D vaccine strain) ⁴
Titer by TCID ₅₀ Assay ^{5,6} in Vero Cells ¹	Report results	8.9 x 10 ⁷ TCID ₅₀ /mL
Functional Activity by RT-PCR Assay Using YFV Specific Primers	~ 350 and 450 bp amplicons	~ 350 and 450 bp amplicons
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
Mycoplasma Contamination Agar and broth culture (14-day incubation at 37°C) DNA Detection by PCR of Test Article nucleic acid	None detected None detected	None detected None detected

Vero cells: ATCC® CCL-81™.

Date: 11 DEC 2007 **Signature:** Signature on File

> Title: Technical Manager, BEI Authentication or designee

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²The inoculum for NR-115 was ATCC® VR-1268 (Lot No. V-525-001-022, NIAID). Cells were grown in Minimum Essential Medium containing Earle's salts and non-essential amino acids (Invitrogen™ 10370-021) supplemented with 2% irradiated fetal bovine serum (Cambrex®14-471E), 2 mM L-glutamine (Invitrogen™ 25030-081), and 1 mM sodium pyruvate (Invitrogen™ 11360-070) for 7 days at 37°C and 5% CO₂.

³Using monoclonal antibody specific to YFV, 17D (Chemicon MAB984).

⁴Also consistent with other strains/isolates of YFV.

 $^{^{5}}$ The 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.

⁶¹⁴ 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.