

Certificate of Analysis for NR-50234

Zika Virus, PLCal_ZV

Catalog No. NR-50234

Product Description: Cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells (Vero 76, clone E6) infected with Zika virus (ZIKV), PLCal_ZV

Passage History: V5/V5¹ (Public Health Agency of Canada/BEI Resources); V# = Number of passages in Vero cells

Lot²: 64103045 Manufacturing Date: 22JUN2016

TEST	SPECIFICATIONS	RESULTS
Infectivity in Vero E6 Cells	Report results	Cell rounding and detachment
Sequencing of Species-Specific Region (1656 nucleotides)	Consistent with ZIKV, PLCal_ZV	99% identity with ZIKV, PLCal_ZV (GenBank: KF993678)
Titer by TCID ₅₀ Assay in Vero E6 Cells ^{3,4}	Report results	1.6 × 10 ⁷ TCID ₅₀ per mL
Sterility (21-day incubation) Harpo's HTYE broth ⁵ , 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

¹Removal of contaminating mycoplasma required three additional virus passages at BEI Resources.

Date: 15 MAR 2017 Signatur

BEI Resources Authentication

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²Grown in Eagle's Minimum Essential Medium containing Earle's Balanced Salt Solution, non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate (ATCC® 30-2003) and 2% fetal bovine serum (ATCC® 30-2020) for 5 days at 37°C with 5% CO₂

³The Tissue Culture Infectious Dose 50% (TCID₅₀) 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.

⁴⁷ days at 37°C with 5% CO₂

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