

Certificate of Analysis for NR-50281

Zika Virus, MEX I-7

Catalog No. NR-50281

This reagent is offered by the Department of Health and Human Services as part of the Public Health Emergency Materials in response to Zika virus.

Product Description: Cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells (Vero 76, clone E6) infected with Zika virus (ZIKV), MEX I-7

Passage History: V4/V4 (Prior to deposit at BEI Resources/BEI Resources); V# = Number of passages in Vero cells¹

Lot²: 64158312 Manufacturing Date: 17MAY2016

TEST	SPECIFICATIONS	RESULTS
Infectivity in Vero E6 Cells	Report results	Refractile cell rounding and detachment
Sequencing of Species-Specific Region (923 nucleotides)	Consistent with ZIKV, MEX I-7	99% identity with ZIKV, MEX I-7 (GenBank: KX247632) ³
Titer by TCID ₅₀ Assay in Vero E6 Cells ^{4,5}	Report results	2.8 x 10 ⁷ TCID ₅₀ per mL
Sterility (21-day incubation) Harpo's HTYE broth ⁶ , 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

¹The second virus passage at BEI Resources was performed by lipofectamine-mediated transfection of extracted viral nucleic acid in order to remove contaminating mycoplasma.

Date: 06 JUL 2016 Signature

BEI Resources Authentication

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

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²Grown in Minimum Essential Medium containing Earle's Balanced Salt Solution (Gibco® 11095-080), supplemented with 1 mM sodium pyruvate (Gibco® 11360-070) and 2% fetal bovine serum (ATCC® 30-2020) for 6 days at 37°C with 5% CO₂

³The complete genomic sequence of NR-50281, Lot No. 64158312 has also been determined (GenBank: KX446951, ZIKV/Aedes.sp/MEX/MEX_I-7/2016).

⁴The 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.
⁵⁸ days at 37°C with 5% CO₂

⁶Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.