Certificate of Analysis for NR-51174

Zika Virus, PRVABC59_BC, Barcoded

Catalog No. NR-51174

Product Description: Zika virus (ZIKV), PRVABC59_BC, barcoded was constructed by introducing a genetic barcode consisting of 8 consecutive degenerate codons into a region of non-structural protein 2a (NS2a; nucleotide position 4008-4031) of the ZIKV, strain PRVABC59.

Lot[1]: 70017964 **Manufacturing Date: 20AUG2018**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero cells[2]	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (~ 830 nucleotides)	≥ 98% identity with ZIKV, PRVABC59 (GenBank: KU501215.1)	100% identity with ZIKV, PRVABC59 (GenBank: KU501215.1)[3]
Confirmation of Barcode in ZIKV NS2a Gene Sequence	Barcode present	Barcode present
Titer by $TCID_{50}$ Assay[4,5] in Vero cells[2] by Cytopathic Effect	Report results	1.6×10^4 $TCID_{50}$ per mL
Amplification of ZIKV Sequence by RT-PCR	~ 1050 base pair amplicon	~ 1050 base pair amplicon
Sterility (21-day incubation)		
Harpo's HTYE broth[6], 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

[1]NR-51174 was produced by amplifying the deposited material using QIAGEN® REPLI-g® kit (catalog No. 150023) under proprietary conditions. Following amplification, the resulting nucleic acid was linearized by restriction enzyme digestion using *Mlu*I and purified. Linearized DNA was used for transfection into human embryonic kidney cells (293T; ATCC® CRL-3216™) using lipofectamine and grown in Dulbecco's Modified Eagle's Medium (ATCC® 30-2002™) supplemented with 2% Fetal Bovine Serum (ATCC® 30-2020™) for 3 days at 37°C and 5% CO_2. For details, please refer to Weger-Lucarelli, J., et al. "Using Barcoded Zika Virus to Assess Virus Population Structure *in vitro* and in *Aedes aegypti* Mosquitoes." Virology. 521 (2018): 138-148. PubMed: 29935423.

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

[3]Sequence does not include the barcoded degenerate eight nucleotides in NS2a gene.

[4]The Tissue Culture Infectious Dose 50% ($TCID_{50}$) is the 50% infectious endpoint in cell culture. The $TCID_{50}$ 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_{50}) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the $TCID_{50}$ provides a measure of the titer (or infectivity) of a virus preparation.

[5]Assay plates were incubated 8 days at 37°C with 5% CO_2.

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

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/Heather Couch/
Heather Couch 21 DEC 2018
Program Manager or designee, ATCC Federal Solutions

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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