b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-9592

Genomic RNA from Japanese Encephalitis Virus, India R53567

Catalog No. NR-9592

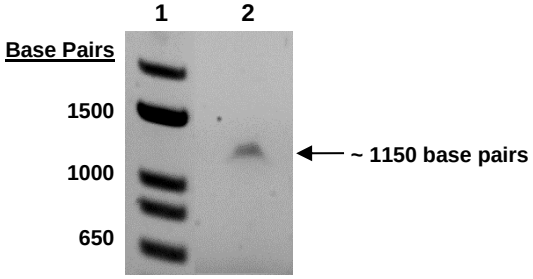
Product Description: Genomic RNA was extracted from a preparation of cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells[1] infected with Japanese encephalitis virus (JEV), India R53567.

Lot[2]: 70010539 **Manufacturing Date: 13NOV2017**

TEST	SPECIFICATIONS	RESULTS
Genotypic Analysis Sequencing of Species-Specific Region (~ 1030 nucleotides)	≥ 98% identity with JEV Consistent with source organism (BEI Resources NR-2332)	98.8% identity with JEV, 826309 polyprotein gene (GenBank: U03689.1)[3] Consistent with NR-2332
Genome Copy Number by ddPCR (Post-Vial)[4]	Report results	2.7 x 10^6 genome copies/mL
Functional Activity by RT-PCR Amplification[5] Glycoprotein gene	~ 1150 base pair amplicon	~ 1150 base pair amplicon (Figure 1)
Pre-Vial Concentration by RiboGreen® Measurement (Viral, Cellular and Carrier)[6]	Report results	359 ng per 100 µL (3.5 µg/mL)
Estimated Amount per Vial[6]	Report Results	359 ng
Virus Inactivation 10% of total yield inoculated on Vero cells[1] and evaluated for cytopathic effect, viral RNA expression and viral antigen expression after serial passage[7]	No viable virus detected	No viable virus detected

[1]Vero cells: ATCC® CCL-81™
[2]Nucleic acid was extracted from a preparation of JEV, India R53567 (BEI Resources NR-2332 lot 58324512), using a QIAamp® Viral RNA Mini Kit (Qiagen 52906).
[3]Sequence information for JEV, India R53567 is not available in the NCBI database; nucleotide sequence obtained for NR-9592 lot 70010539 is highly similar to several JEV strains.
[4]ddPCR data was obtained post-vial from 9 replicates on the BioRad QX200 Droplet Digital PCR (ddPCR™) System
[5]Amplified using iTaq™ Universal SYBR Green One-step Kit (Bio-Rad 172-5151) with 5 µL of NR-9592 in a 50 µL reaction
[6]Measurement is determined pre-vial prior to dilution due to the limit of detection of the quantification method.
[7]Use of the QIAamp® Viral RNA Mini Kit has been demonstrated to consistently inactivate 100% Japanese encephalitis viruses as shown by the absence of cytopathic effect, viral RNA expression by RT-PCR and viral antigen expression by indirect immunofluorescence using Anti-Flavivirus group antigen antibody (BEI Resources NR-50327) after plating the entire extract on virus-susceptible cells.

Figure 1: Functional Activity of NR-9592 by RT-PCR Amplification of Glycoprotein gene



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
Lane 2: PCR product from 1 µL of NR-9592

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/Heather Couch/
Heather Couch 06 SEP 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.

Support Provided by NIAID

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