SUPPORTING INFECTIOUS DISEASE RESEARCH

Genomic RNA from SARS-Related Coronavirus 2, Isolate USA-CA3/2020

Catalog No. NR-52507

This reagent is the property of the U.S. Government.

Product Description:

Genomic RNA was extracted from a preparation of cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells (Vero E6; ATCC[®] CRL-1586[™]) infected with severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate USA-CA3/2020 (BEI Resources NR-52385 lot 70034883), using QIAamp[®] Viral RNA Mini Kit (Qiagen 52904). The viral genomic RNA is in a background of cellular nucleic acid and carrier RNA.

Lot: 70035258

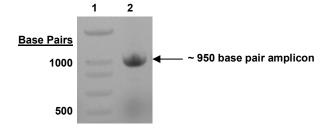
Manufacturing Date: 21MAY2020

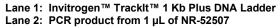
TEST	SPECIFICATIONS	RESULTS		
Sequencing of Species-Specific Region (~ 870 nucleotides)	≥ 98% identity with SARS-CoV-2, isolate USA-CA3/2020 (GenBank: MT027062.1)	100% identity with SARS-CoV-2, isolate USA-CA3/2020 (GenBank: MT027062.1)		
Next-Generation Sequencing (NGS) of Complete Genome Using Illumina [®] iSeq™ 100 Platform (Refer to Appendix I for NGS information)	≥ 98% identity with SARS-CoV-2, isolate USA-CA3/2020 (GenBank: MT027062.1)	99.99% identity with SARS-CoV-2, isolate USA-CA3/2020 (GenBank: MT027062.1)		
Functional Activity by RT-PCR Amplification ¹	~ 950 base pair amplicon	~ 950 base pair amplicon (Figure 1)		
Genome Copy Number Using BioRad QX200 Droplet Digital PCR (ddPCR™) System (Post vial; 5 replicates)	Report results	1.87 × 10 ⁸ genome equivalents/mL		
Virus Inactivation (14 Days, 2 Passages) 10% of total yield inoculated on Vero E6 cells and evaluated for cytopathic effect ²				
Passage 1	No viable virus detected	No viable virus detected		
Passage 2	No viable virus detected	No viable virus detected		
Virus Inactivation (14 Days, 2 Passages) 100% of total yield inoculated on Vero E6 cells and evaluated for cytopathic effect ²				
Passage 1	No viable virus detected	No viable virus detected		
Passage 2	No viable virus detected	No viable virus detected		

¹Amplified using iTaq™ Universal SYBR Green One-step Kit (Bio-Rad 172-5151) with 5 μL of NR-52507 in a 50 μL reaction

²Supernatant was tested by qPCR to confirm absence of CPE and no evidence of replicative RNA was detected. Samples from both passages were tested by qPCR at the end of day 14 of the passages.

Figure 1: Functional Activity of NR-52507 by RT-PCR Amplification





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Certificate of Analysis for NR-52507

/Heather Couch/ Heather Couch

18 SEP 2020

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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APPENDIX I: NGS Information for NR-52507 lot 70035258

Sequence analysis resulted in the discovery of four SNPs when compared to GenBank MT027062.1 (see Table below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Position in NR-52507_ 70035258 Sequence	Position in MT027062.1	Reported MT027062.1 Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
11072	11083	G	Т	225	SNP	1	1.0000000
21757	21768	А	G	82	SNP	1	0.1943320
21765	21776	G	A	123	SNP	1	0.2064777
21773	21784	Т	A	42	SNP	1	0.1752988