

Certificate of Analysis for NR-52503

Genomic RNA from SARS-Related Coronavirus 2, Isolate USA-IL1/2020

Catalog No. NR-52503

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Product Description:

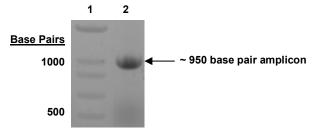
Genomic RNA was extracted from a preparation of cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells (Vero E6; ATCC® CRL-1586™) infected with severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate USA-IL1/2020 (BEI Resources NR-52381 lot 70034875), using QIAamp® Viral RNA Mini Kit (Qiagen 52904). The viral genomic RNA is in a background of cellular nucleic acid and carrier RNA.

Lot: 70035254 Manufacturing Date: 21MAY2020

TEST	SPECIFICATIONS	RESULTS		
Sequencing of Species-Specific Region (~ 870 nucleotides)	≥ 98% identity with SARS-CoV-2, isolate USA-IL1/2020 (GenBank: MN988713.1)	100% identity with SARS-CoV-2, isolate USA-IL1/2020 (GenBank: MN988713.1)		
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-IL1/2020 (GenBank: MN988713.1)	99.92% identity with SARS-CoV-2, isolate USA-IL1/2020 (GenBank: MN988713.1)		
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; 9 replicates)	Report results	5.92 × 10 ⁷ 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-52503 in a 50 μL reaction.

Figure 1: Functional Activity of NR-52503 by RT-PCR Amplification



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder Lane 2: PCR product from 1 µL of NR-52503

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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.



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/Heather Couch/ Heather Couch

18 SEP 2020

Program Manager or designee, ATCC Federal Solutions

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APPENDIX I: NGS Information for NR-52503 lot 70035254

Sequence analysis resulted in the discovery of ten SNPs and one deletion when compared to GenBank MN988713.1 (see Table below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Position in NR-52503_ 70035254 Sequence	Position in MN988713.1	Reported MN988713.1 Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
486	490	W	A	225	SNP	1	1.0000000
3173	3177	Y	Т	225	SNP	1	1.0000000
8778	8782	Y	Т	225	SNP	1	1.0000000
9514	9518	Т	С	228	SNP	1	0.9957082
22339	22343	G	С	179	SNP	1	0.2622951
23581	23585	CAGACTCAGACTAATT	С	222	Indel	15	0.6134454
24015	24034	Y	Т	225	SNP	1	1.0000000
26710	26729	Y	С	225	SNP	1	1.0000000
28058	28077	S	С	225	SNP	1	1.0000000
28125	28144	Y	С	225	SNP	1	1.0000000
28835	28854	Υ	С	225	SNP	1	1.0000000

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