

Genomic RNA from SARS-Related Coronavirus 2, Isolate Chile/Santiago_op4d1/2020

Catalog No. NR-52510

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 Chile/Santiago_op4d1/2020 (BEI Resources NR-52439 lot 70035562), using QIAamp® Viral RNA Mini Kit (Qiagen 52904). The viral genomic RNA is in a background of cellular nucleic acid and carrier RNA.

Lot: 70037428

Manufacturing Date: 16JUN2020

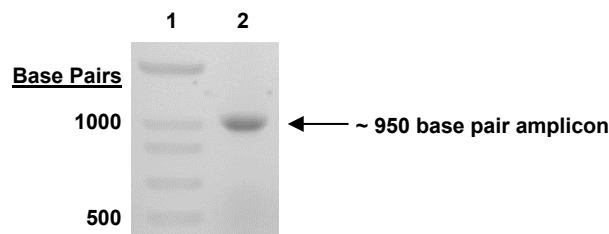
TEST	SPECIFICATIONS	RESULTS
Sequencing of Species-Specific Region (~ 870 nucleotides)	≥ 98% identity with SARS-CoV-2, isolate Chile/Santiago_op4d1/2020 (GISAID: EPI_ISL_415661)	100% identity with SARS-CoV-2, isolate Chile/Santiago_op4d1/2020 (GISAID: EPI_ISL_415661)
Next-Generation Sequencing (NGS) of Complete Genome Using Illumina® iSeq™ 100 Platform (Refer to Appendix I for NGS information)	≥ 98% identity with SARS-CoV-2, isolate Chile/Santiago_op4d1/2020 (GISAID: EPI_ISL_415661)	100% identity with SARS-CoV-2, isolate Chile/Santiago_op4d1/2020 (GISAID: EPI_ISL_415661)
Functional Activity by RT-PCR Amplification¹	~ 950 base pair amplicon	~ 950 base pair amplicon (Figure 1)
Estimated Concentration (post-dilution) by RiboGreen® Measurement (Viral, Cellular and Carrier)²	Report results	8.8 ng per 100 µL (0.09 µg/mL)
Estimated Amount per Vial²	Report results	8.8 ng
Genome Copy Number Using BioRad QX200 Droplet Digital PCR (ddPCR™) System (Post vial; 9 replicates)	Report results	6.33 × 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 ³ Passage 1 Passage 2	No viable virus detected No viable virus detected	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 ³ Passage 1 Passage 2	No viable virus detected No viable virus detected	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-52510 in a 50 µL reaction

²Measurement is determined pre-vial prior to dilution due to the limit of detection of the quantification method

³Supernatant 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-52510 by RT-PCR Amplification



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
Lane 2: PCR product from 1 µL of NR-52510

/Heather Couch/
Heather Couch

25 JAN 2021

Program Manager or designee, ATCC Federal Solutions

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APPENDIX I: NGS Information for NR-52510 lot 70037428

Sequence analysis resulted in the discovery of four SNPs when compared to the reference sequence from GISAID EPI_ISL_415661. Additionally, both the reference sequence GISAID EPI_ISL_415661 and NR-52510_70037428 contained seven SNPs when compared to GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) (see Table below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Position in NR-52510_70037428 Sequence	Position in EPI_ISL_415661 Reference Sequence	Position in MN908947 Sequence	Reported MN908947 Sequence	Reported EPI_ISL_415661 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
8727	8727	8782	C	T	T	N/A	SNP	1	1.0000000
9422	9422	9477	T	A	A	N/A	SNP	1	1.0000000
10590	10590	10645	C	C	T	228	SNP	1	0.9773371
14750	14750	14805	C	T	T	N/A	SNP	1	1.0000000
22062	22062	22117	T	T	G	221	SNP	1	0.2745826
22241	22241	22296	A	A	G	221	SNP	1	0.2822384
23561	23561	23616	G	G	A	228	SNP	1	0.9888211
25924	25924	25979	G	T	T	N/A	SNP	1	1.0000000
23089	23089	28144	T	C	C	N/A	SNP	1	1.0000000
28602	28602	28657	C	T	T	N/A	SNP	1	1.0000000
28808	28808	28863	C	T	T	N/A	SNP	1	1.0000000