

Genomic RNA from SARS-Related Coronavirus 2, Isolate Hong Kong/VM20001061/2020

Catalog No. NR-52388

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 Hong Kong/VM20001061/2020 (BEI Resources NR-52282 lot 70034432), using QIAamp® Viral RNA Mini Kit (Qiagen 52904). The viral genomic RNA is in a background of cellular nucleic acid and carrier RNA.

Lot: 70034679

Manufacturing Date: 06APR2020

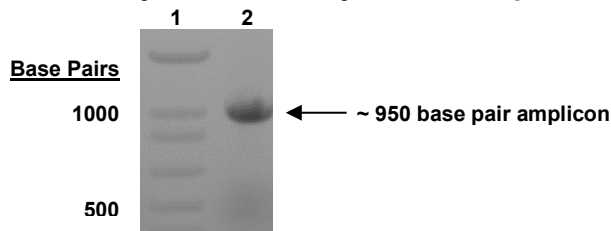
TEST	SPECIFICATIONS	RESULTS
Next-Generation Sequencing (NGS) of Complete Genome Using Illumina® iSeq™ 100 Platform (Refer to Appendix I for NGS information)	≥ 98% identity with SARS-CoV-2, isolate hCoV-19/Hong Kong/VM20001061-2/2020 (GISAID: EPI_ISL_412028)	99.9% identity with SARS-CoV-2, isolate hCoV-19/Hong Kong/VM20001061-2/2020 (GISAID: EPI_ISL_412028)
Sequencing of Species-Specific Region (~ 880 nucleotides)	≥ 98% identity with SARS-CoV-2, isolate SARS-CoV-2/human/USA/NR-52282/2020 (GenBank: MT547814.1)	100% identity with SARS-CoV-2, isolate SARS-CoV-2/human/USA/NR-52282/2020 (GenBank: MT547814.1)
Functional Activity by RT-PCR Amplification¹	~ 950 base pair amplicon	~ 950 base pair amplicon (Figure 1)
Pre-Vial Concentration by RiboGreen® Measurement (Viral, Cellular and Carrier)²	Report results	28.8 ng per 100 µL (0.29 µg/mL)
Estimated Amount per Vial³	Report results	28.8 ng
Genome Copy Number Using BioRad QX200 Droplet Digital PCR (ddPCR™) System (Post vial; 9 replicates)	Report results	4.82 × 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-52388 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-52388 by RT-PCR Amplification



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
Lane 2: PCR product from 1 µL of NR-52388

/Heather Couch/
Heather Couch

09 SEP 2020

Program Manager or designee, ATCC Federal Solutions

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APPENDIX I: NGS Information for NR-52388 lot 70034679

Sequence analysis resulted in the discovery of seven SNPs and one deletion (indel) when compared to the reference sequence from GISAID EPI_ISL_412028. Additionally, both the reference sequence GISAID EPI_ISL_412028 and NR-52388_70034679 contained six 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-52388_70034679 Sequence	Position in EPI_ISL_412028 Reference Sequence	Position in MN908947 Sequence	Reported MN908947 Sequence	Reported EPI_ISL_412028 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
1650	1650	1663	C	T	T	n/a	SNP	1	1.0000000
8769	8769	8782	C	T	T	n/a	SNP	1	1.0000000
12906	12906	12919	C	C	T	222	SNP	1	0.6201117
21623	21623	21636	C	C	T	219	SNP	1	0.8716578
22648	22648	22661	G	T	T	n/a	SNP	1	1.0000000
23594	23594	23607	G	G	A	228	SNP	1	0.9578947
24021	24021	24034	C	Y	T	225	SNP	1	1.0000000
24553	24553	24566	C	C	G	221	SNP	1	0.7255814
26716	26716	26729	T	C	C	n/a	SNP	1	1.0000000
27250	27250	27263	CTTTTAAA GTTTCCAT TTGGAAT CTTGATT	CTTTTAAA GTTTCCAT TTGGAAT CTTGATT	CTT	221	Indel	27	0.7064220
28037	28064	28077	G	C	C	n/a	SNP	1	1.0000000
28104	28131	28144	T	C	C	n/a	SNP	1	1.0000000
29822	29849	29862	G	T	G	141	SNP	1	1.0000000