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SUPPORTING INFECTIOUS DISEASE RESEARCH

Genomic RNA from SARS-Related Coronavirus 2, Isolate USA-CA4/2020

Catalog No. NR-52508

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-CA4/2020 (BEI Resources NR-52386 lot 70034885), using QIAamp® Viral RNA Mini Kit (Qiagen® 52904). The viral genomic RNA is in a background of cellular nucleic acid and carrier RNA.

Lot: 70035259   Manufacturing Date: 28MAY2020

TEST	SPECIFICATIONS	RESULTS
Sequencing of Species-Specific Region (860 nucleotides)	≥ 98% identity with SARS-CoV-2, isolate USA-CA4/2020 (GenBank: MT027063.1)	100% identity with SARS-CoV-2, isolate USA-CA4/2020 (GenBank: MT027063.1)
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-CA4/2020 (GenBank: MT027063.1)	99.99% identity with SARS-CoV-2, isolate USA-CA4/2020 (GenBank: MT027063.1)
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; 6 replicates)	Report results	2.67×10^8 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 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[2] Passage 1 Passage 2	No viable virus detected No viable virus detected	No viable virus detected No viable virus detected

[1] Amplified using iTaq™ Universal SYBR Green One-step Kit (Bio-Rad® 172-5151) with 5 µL of NR-52508 in a 50 µL reaction
[2] Supernatant 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-52508 by RT-PCR Amplification
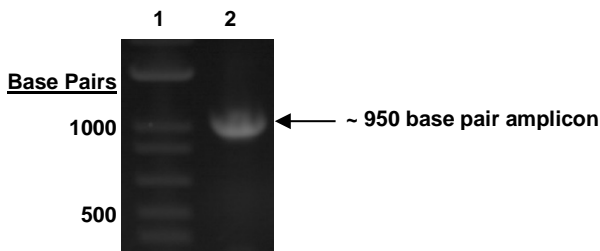
Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
Lane 2: PCR product from 1 µL of NR-52508

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/Heather Couch/

Heather Couch   08 OCT 2020

Program Manager or designee, ATCC Federal Solutions

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APPENDIX I: NGS Information for NR-52508 lot 70035259

Sequence analysis resulted in the discovery of three SNPs when compared to GenBank MT027063.1 (see Table below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Position in NR-52508_ 70035259 Sequence	Position in MT027063.1	Reported MT027063.1 Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
22195	22206	A	G	53	SNP	1	0.1889764
23596	23607	G	T	221	SNP	1	0.8170213
26187	26198	C	T	221	SNP	1	0.7892562