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SUPPORTING INFECTIOUS DISEASE RESEARCH

# Certificate of Analysis for NR-52505

## Genomic RNA from SARS-Related Coronavirus 2, Isolate USA-AZ1/2020

## Catalog No. NR-52505

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-AZ1/2020 (BEI Resources NR-52383 lot 70034879), using QIAamp® Viral RNA Mini Kit (Qiagen 52904). The viral genomic RNA is in a background of cellular nucleic acid and carrier RNA.

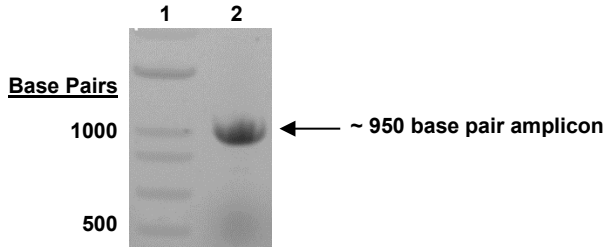
**Lot: 70035256** **Manufacturing Date: 20MAY2020**

| TEST | SPECIFICATIONS | RESULTS |
|---|---|---|
| **Sequencing of Species-Specific Region** (~ 870 nucleotides) | ≥ 98% identity with SARS-CoV-2, isolate USA-AZ1/2020 (GenBank: MN997409.1) | 100% identity with SARS-CoV-2, isolate USA-AZ1/2020 (GenBank: MN997409.1) |
| **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-AZ1/2020 (GenBank: MN997409.1) | 99.92% identity with SARS-CoV-2, isolate USA-AZ1/2020 (GenBank: MN997409.1) |
| **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 | $6.78 \times 10^7$ 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 |

[1]Amplified using iTaq™ Universal SYBR Green One-step Kit (Bio-Rad 172-5151) with 5 µL of NR-52505 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-52505 by RT-PCR Amplification**




**Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder**
**Lane 2: PCR product from 1 µL of NR-52505**

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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.



Support Provided by NIAID

## APPENDIX I: NGS Information for NR-52505 lot 70035256

Sequence analysis resulted in the discovery of one SNP and one deletion when compared to GenBank MN997409.1 (see Table 1 below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

| Position in NR-52505_ 70035256 Sequence | Position in MN997409.1 | Reported MN997409.1 Sequence | Identified Alternative Base | Quality | Variant Type | Length of Variant | Frequency of Variant |
|---|---|---|---|---|---|---|---|
| 15533 | 15540 | C | T | 203 | SNP | 1 | 0.2400000 |
| 23593 | 23600 | TCTCCTCGGCGGGCACGT AGTGTAGCT | TCT | 228 | Indel | 24 | 0.8613445 |

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