

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

Catalog No. NR-52501

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 Singapore/2/2020 (BEI Resources NR-52369 lot 70035611), using QIAamp® Viral RNA Mini Kit (Qiagen 52904). The viral genomic RNA is in a background of cellular nucleic acid and carrier RNA.

Lot: 70037086

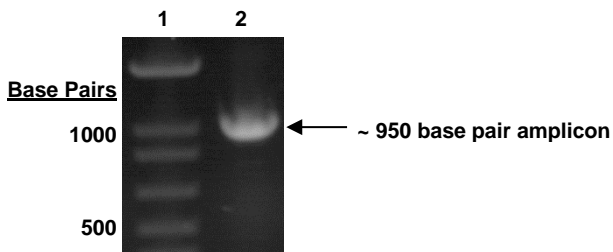
Manufacturing Date: 20MAY2020

| TEST | SPECIFICATIONS | RESULTS |
|---|---|--|
| Sequencing of Species-Specific Region (~ 930 nucleotides) | ≥ 98% identity with SARS-CoV-2, isolate Singapore/2/2020 (GISAID: EPI_ISL_407987) | 100% identity with SARS-CoV-2, isolate Singapore/2/2020 (GISAID: EPI_ISL_407987) |
| 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 Singapore/2/2020 (GISAID: EPI_ISL_407987) | 99.97% identity with SARS-CoV-2, isolate Singapore/2/2020 (GISAID: EPI_ISL_407987) |
| Functional Activity by RT-PCR Amplification¹ | ~ 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 | 5.71 × 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 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 |

¹Amplified using iTaq™ Universal SYBR Green One-step Kit (Bio-Rad 172-5151) with 5 µL of NR-52501 in a 50 µL reaction

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.

Figure 1: Functional Activity of NR-52501 by RT-PCR Amplification



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
Lane 2: PCR product from 1 µL of NR-52501

/Heather Couch/

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05 OCT 2020

Program Manager or designee, ATCC Federal Solutions

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APPENDIX I: NGS Information for NR-52501 lot 70037086

Sequence analysis resulted in the discovery of five SNPs and one deletion when compared to the reference sequence from GISAID EPI_ISL_407987 (see Table below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

| Position in NR-52501_70037086 Sequence | Position in EPI_ISL_407987 Reference Sequence | Reported EPI_ISL_407987 Reference Sequence | Identified Alternative Base | Quality | Variant Type | Length of Variant | Frequency of Variant |
|--|---|--|-----------------------------|---------|--------------|-------------------|----------------------|
| 507 | 516 | TATG | T | N/A | Indel | 3 | 0.5660127 |
| 22084 | 22096 | C | T | 104 | SNP | 1 | 0.2127660 |
| 22085 | 22097 | T | C | 108 | SNP | 1 | 0.2145923 |
| 22087 | 22099 | G | A | 104 | SNP | 1 | 0.2127660 |
| 22283 | 22295 | A | G | 186 | SNP | 1 | 0.2827869 |
| 23603 | 23615 | G | A | 228 | SNP | 1 | 0.9036697 |