

**Genomic RNA from SARS-Related Coronavirus 2, Isolate England/02/2020**

**Catalog No. NR-52499**

**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 England/02/2020 (BEI Resources NR-52359 lot 70036177), using QIAamp® Viral RNA Mini Kit (Qiagen® 52904). The viral genomic RNA is in a background of cellular nucleic acid and carrier RNA.

**Lot: 70037085**

**Manufacturing Date: 10JUN2020**

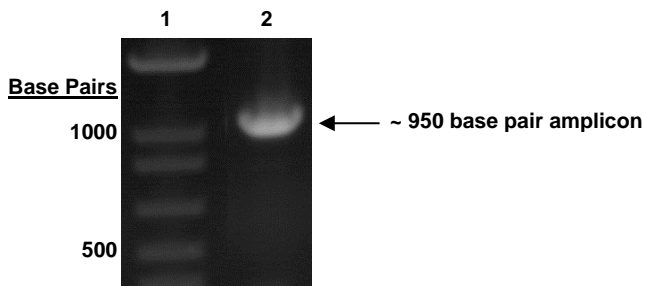
TEST	SPECIFICATIONS	RESULTS
<b>Sequencing of Species-Specific Region</b> (~ 840 nucleotides)	≥ 98% identity with SARS-CoV-2, isolate England/02/2020 (GISAID: EPI_ISL_407073)	100% identity with SARS-CoV-2, isolate England/02/2020 (GISAID: EPI_ISL_407073)
<b>Next-Generation Sequencing (NGS) of Complete Genome Using Illumina® iSeq™ 100 Platform</b> (Refer to Appendix I for NGS information)	≥ 98% identity with SARS-CoV-2	≥ 98% identity with SARS-CoV-2 <sup>1</sup>
<b>Functional Activity by RT-PCR Amplification<sup>2</sup></b>	~ 950 base pair amplicon	~ 950 base pair amplicon (Figure 1)
<b>Genome Copy Number Using BioRad QX200 Droplet Digital PCR (ddPCR™) System</b> (Post vial; 9 replicates)	Report results	6.5 x 10 <sup>7</sup> genome equivalents/mL
<b>Virus Inactivation (14 Days, 2 Passages)</b> 10% of total yield inoculated on Vero E6 cells and evaluated for cytopathic effect <sup>3</sup> Passage 1 Passage 2	No viable virus detected No viable virus detected	No viable virus detected No viable virus detected
<b>Virus Inactivation (14 Days, 2 Passages)</b> 100% of total yield inoculated on Vero E6 cells and evaluated for cytopathic effect <sup>3</sup> Passage 1 Passage 2	No viable virus detected No viable virus detected	No viable virus detected No viable virus detected

<sup>1</sup>Sequence information for SARS-CoV-2, England/02/2020 is not available in the NCBI database; nucleotide sequence obtained for NR-52499 lot 70037085 is 99.9% identical to SARS-CoV-2 isolate Wuhan-Hu-1, complete genome (GenBank: MN908947.3) and consistent with numerous SARS-CoV-2 strains.

<sup>2</sup>Amplified using iTaq™ Universal SYBR Green One-step Kit (Bio-Rad® 172-5151) with 5 µL of NR-52499 in a 50 µL reaction

<sup>3</sup>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-52499 by RT-PCR Amplification**



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder  
Lane 2: PCR product from 1 µL of NR-52499

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21 OCT 2020

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**APPENDIX I: NGS Information for NR-52499 lot 70037085**

Sequence analysis resulted in the discovery of six SNPs and two deletions when compared to SARS-CoV-2 isolate Wuhan-Hu-1, complete genome (GenBank: MN908947.3) (see Table below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Position in NR-52499_70037085 Sequence	Position in MN908947.3	Reported MN908947.3 Sequence	Identified Alternative Base	Quality	Variant Type	Frequency of Variant
673	685	AAAGTCATTTGACTTA	AGACTTA	61	Indel	0.108787
7737	7749	C	T	222	SNP	0.326667
8216	8228	C	T	221	SNP	0.283186
8770	8782	C	T	225	SNP	1.000000
18476	18488	T	C	225	SNP	1.000000
23585	23597	AATTCTCCTCGGCGGG CACGTAGTG	A	228	Indel	0.824121
28108	28144	T	C	225	SNP	1.000000
29560	29596	A	G	225	SNP	1.000000