

Genomic RNA from SARS-Related Coronavirus 2, Isolate Germany/BavPat1/2020

Catalog No. NR-52502

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 Germany/BavPat1/2020 (BEI Resources NR-52370 lot 70036595), using QIAamp® Viral RNA Mini Kit (Qiagen 52904). The viral genomic RNA is in a background of cellular nucleic acid and carrier RNA.

Lot: 70036181

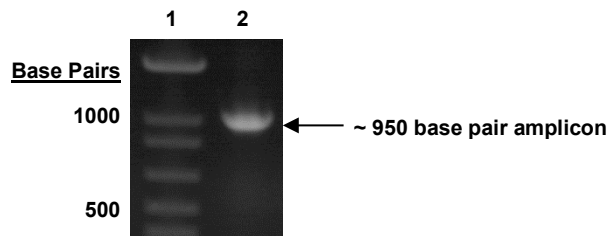
Manufacturing Date: 16JUN2020

TEST	SPECIFICATIONS	RESULTS
Sequencing of Species-Specific Region (810 nucleotides)	≥ 98% identity with SARS-CoV-2, isolate Human/DEU/BavPat1-ChVir929/2020 (GenBank: MT270101.1)	100% identity with SARS-CoV-2, isolate Human/DEU/BavPat1-ChVir929/2020 (GenBank: MT270101.1)
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 Human/DEU/BavPat1-ChVir929/2020 (GenBank: MT270101.1 and GISAID: EPI_ISL_406862)	99.93% identity with SARS-CoV-2, isolate Human/DEU/BavPat1-ChVir929/2020 (GenBank: MT270101.1 and GISAID: EPI_ISL_406862)
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; 6 replicates)	Report results	1.09 × 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

¹Amplified using iTaq™ Universal SYBR Green One-step Kit (Bio-Rad 172-5151) with 5 µL of NR-52502 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-52502 by RT-PCR Amplification



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
Lane 2: PCR product from 1 µL of NR-52502

/Heather Couch/
Heather Couch

18 SEP 2020

Program Manager or designee, ATCC Federal Solutions

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APPENDIX I: NGS Information for NR-52502 lot 70036181

Sequence analysis resulted in the discovery of four SNPs and one deletion when compared to the reference sequence from GISAID EPI_ISL_406862. Additionally, both the reference sequence GISAID EPI_ISL_406862 and NR-52502_70036181 contained three 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-52502_70036181 Sequence	Position in EPI_ISL_406862 Reference Sequence	Position in MN908947 Sequence	Reported MN908947 Sequence	Reported EPI_ISL_406862 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
187	187	241	C	T	T	n/a	SNP	1	1.0000000
2983	2983	3037	C	T	T	n/a	SNP	1	1.0000000
5752	5752	5806	C	C	T	222	SNP	1	0.6200397
13939	13939	13993	G	G	T	228	SNP	1	0.9842342
21952	21952	22006	C	C	A	51	SNP	1	0.1939655
23349	23349	23403	A	G	G	n/a	SNP	1	1.0000000
23541	23541	23595	CTAATTCT CCTTCGG CGGGCAC	CTAATTCT CCTTCGGC GGGCAC	C	n/a	Indel	20	0.5647637
28797	28814	28868	C	C	T	228	SNP	1	0.9840286