

SARS-Related Coronavirus 2, Isolate hCoV-19/South Africa/KRISP-K020179/2021, clone PP6 (Lineage C.1.2)

Catalog No. NR-56327

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/South Africa/KRISP-K020179/2021, clone PP6 was plaque-purified after one passage at Emory University in January 2022. NR-56327 lot 70048886 was produced by infecting *Homo sapiens* lung adenocarcinoma epithelial cells (Calu-3; ATCC® HTB-55™) with the deposited material and incubating in Eagle's Minimum Essential Medium (ATCC® 30-2003™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) for 3 days at 37°C with 5% CO₂. The cells and supernatant were spin-clarified at 1500 × g for 10 minutes at 4°C.

Passage History:

VT(1)/C(1) (Emory University/BEI Resources); VT = *Cercopithecus aethiops* kidney cells with transmembrane protease, serine 2 gene (Vero E6-TMPRSS2); C = Calu-3

Lot: 70048886

Manufacturing Date: 15NOV2021

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TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Calu-3 Cells	Cell rounding and detachment	Cell rounding and detachment
Next-Generation Sequencing (NGS) of Complete Genome Using Illumina® iSeq™ 100 Platform (Refer to Appendix I for NGS information)	≥ 98% identity with isolate hCoV-19/South Africa/KRISP-K020179/2021, clone PP6 (GISAID: EPI_ISL_3267757)	99.85% identity with isolate hCoV-19/South Africa/KRISP-K020179/2021, clone PP6 (GISAID: EPI_ISL_3267757)
Titer by TCID₅₀ Assay in Calu-3 Cells by Cytopathic Effect¹ (7 days at 37°C and 5% CO ₂)	Report results	2.0 × 10 ⁶ TCID ₅₀ per mL ²
Sterility (21-day incubation) Harpo's HTYE broth, 37°C and 26°C, aerobic ³ Trypticase Soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C, aerobic	No growth No growth No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth No growth No growth
Mycoplasma Contamination Agar and broth culture (14-day incubation at 37°C) DNA detection by PCR of extracted Test Article nucleic acid	None detected None detected	None detected None detected

¹The Tissue Culture Infectious Dose 50% (TCID₅₀) endpoint is the 50% infectious endpoint in cell culture. The TCID₅₀ is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the culture vessels inoculated, just as a Lethal Dose 50% (LD₅₀) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID₅₀ provides a measure of the titer (or infectivity) of a virus preparation.

²Titer was determined by cytopathic effects (CPE) and completed in triplicate (1.6 × 10⁶ per mL, 1.6 × 10⁶ per mL and 2.8 × 10⁶ per mL). The average of the three values is reported.

³Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

/Sonia Bjorun Brower/

Sonia Bjorun Brower

Technical Manager or designee, ATCC Federal Solutions

31 AUG 2022

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APPENDIX I: NGS Information for NR-56327 lot 70048886

Note: The sequence submitted to GISAID for this isolate (EPI_ISL_3267757) contains stretches of ambiguous nucleotides (Ns), which required the selection of an alternate sequence to serve as a suitable reference for comparison of the sequence from NR-56327. The reference sequence used for quality control testing and reporting purposes is the sequence of the plaque pick virus material submitted to BEI Resources. The sequence is available upon request.

Sequence analysis using AMGP readsQC-illumina.py pipeline and variant callers LoFreq version: 2.1.5 and freebayes version: v1.3.1-dirty showed that both the provided reference sequence and NR-56327 lot 70048886 contained forty-eight SNPs and five deletions (DEL) when compared to GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) (see Table I below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Table I: Variants with different nucleotides between NR-56327 lot 70048886 and GenBank MN908947.3 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome)

Variant Type	Variant Position and Identified Alternative Base	Coverage ¹	Length of Variant	Frequency of Variant ¹	Gene (Region)	Amino Acid Mutation
SNP	c241t	N/A	1	100.0000%	5'UTR	Untranslated
SNP	g569a	N/A	1	100.0000%	ORF1ab (nsp1)	E102K
SNP	c703t	N/A	1	100.0000%	ORF1ab (nsp1)	Silent mutation
SNP	c745t	N/A	1	100.0000%	ORF1ab (nsp1)	Silent mutation
SNP	c2485t	N/A	1	100.0000%	ORF1ab (nsp2)	Silent mutation
SNP	c3037t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c4002t	N/A	1	100.0000%	ORF1ab (nsp3)	T428I
SNP	c5175t	N/A	1	100.0000%	ORF1ab (nsp3)	T819I
SNP	a7699g	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	g10097a	N/A	1	100.0000%	ORF1ab (nsp5)	G15S
SNP	c10125t	N/A	1	100.0000%	ORF1ab (nsp5)	T24I
DEL	Δ11288-11296	N/A	-9	100.0000%	ORF1ab (nsp6)	ΔSGF (amino acids 106-108)
SNP	c13019t	N/A	1	100.0000%	ORF1ab (nsp9)	Silent mutation
SNP	c13536t	N/A	1	100.0000%	ORF1ab (nsp12)	Silent mutation
SNP	c14408t	N/A	1	100.0000%	ORF1ab (nsp12)	P323L
SNP	g17562t	N/A	1	100.0000%	ORF1ab (nsp13)	Silent mutation
SNP	c17703t	N/A	1	100.0000%	ORF1ab (nsp13)	Silent mutation
SNP	c18747t	N/A	1	100.0000%	ORF1ab (nsp14)	Silent mutation
SNP	t19053c	N/A	1	100.0000%	ORF1ab (nsp14)	Silent mutation
SNP	c21588t	N/A	1	100.0000%	Spike	P9L
SNP	g21969t	N/A	1	100.0000%	Spike	C136F

Variant Type	Variant Position and Identified Alternative Base	Coverage ¹	Length of Variant	Frequency of Variant ¹	Gene (Region)	Amino Acid Mutation
DEL	Δ21991-21993	N/A	-3	100.0000%	Spike	ΔY (amino acids 143-144)
SNP	g22132t	N/A	1	100.0000%	Spike	R190S
SNP	a22206g	N/A	1	100.0000%	Spike	D215G
SNP	c22210t	N/A	1	100.0000%	Spike	Silent mutation
DEL	Δ22289-22294	N/A	-6	100.0000%	Spike	ΔAL (amino acids 243-244)
SNP	t22907c	N/A	1	100.0000%	Spike	Y449H
SNP	g23012a	N/A	1	100.0000%	Spike	E484K
SNP	a23063t	N/A	1	100.0000%	Spike	N501Y
SNP	a23403g	N/A	1	100.0000%	Spike	D614G
SNP	c23525t	N/A	1	100.0000%	Spike	H655Y
SNP	t23599a	N/A	1	100.0000%	Spike	N679K
SNP	c23709t	N/A	1	100.0000%	Spike	T716I
SNP	c23731t	N/A	1	100.0000%	Spike	Silent mutation
SNP	c24138a	N/A	1	100.0000%	Spike	T859N
SNP	c26078a	N/A	1	100.0000%	ORF3a	T229N
DEL	Δ26155-26157	N/A	-3	100.0000%	ORF3a	ΔV255
SNP	c26305a	N/A	1	100.0000%	Envelope	L21I
SNP	c26607t	N/A	1	100.0000%	Membrane	L29F
SNP	t26767c	N/A	1	100.0000%	Membrane	I82T
DEL	Δ27792-27793	N/A	-2	100.0000%	ORF7b	LAFLLFLVLIIMLIIFWFSL amino acids 14-31 SLSAIPCFNYAYLLVLT Δamino acids 32-43 ²
SNP	c27879t	N/A	1	100.0000%	ORF7b	H42Y
SNP	c27945t	N/A	1	100.0000%	ORF8	Q18*
SNP	g28086t	N/A	1	100.0000%	ORF8	A65S
SNP	a28272t	N/A	1	100.0000%	Intergenic – ORF8/Nucleocapsid	Untranslated
SNP	c28311t	N/A	1	100.0000%	Nucleocapsid	P13L
SNP	t28516c	N/A	1	100.0000%	Nucleocapsid	Silent mutation
SNP	c28603t	N/A	1	100.0000%	Nucleocapsid	Silent mutation
SNP	t28829c	N/A	1	100.0000%	Nucleocapsid	S186P
SNP	g28881a	N/A	1	100.0000%	Nucleocapsid	R203K
SNP	g28882a	N/A	1	100.0000%		
SNP	g28883c	N/A	1	100.0000%	Nucleocapsid	G204R
SNP	g29425t	N/A	1	100.0000%	Nucleocapsid	Q384H

¹Coverage for all variants in Table I is listed as 'N/A'. There is no read coverage information for these variants because the sample reads are only mapped to the reference sequence and not to the SARS-CoV-2, Wuhan-Hu-1 isolate sequence (GenBank MN908947), but that does not mean these areas lack for coverage. All variants in Table I are mismatches between the reference sequence and the SARS-CoV-2, Wuhan-Hu-1 sequence, so these variants will be assigned a frequency of 100% and will therefore be majority SNPs.

²Due to the deletion Δ27792-27793, the amino acids from 14 to 31 are changed from LAFLLFLVLIIMLIIFWFSL to SLSAIPCFNYAYLLVLT. Amino acids 32 to 43 are deleted.