

SARS-Related Coronavirus 2, Isolate hCoV-19/South Africa/CERI-KRISP-K040013/2022 (Lineage BA.5; Omicron Variant)

Catalog No. NR-56798

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/South Africa/CERI-KRISP-K040013/2022 was isolated from a human on April 20, 2022, in Durban, eThekweni, South Africa. NR-56798 lot 70053884 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 4 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:

V(2)/C(1) (Africa Health Research Institute/BEI Resources); V = *Cercopithecus aethiops* kidney cells (Vero); C = Calu-3

Lot: 70053884

Manufacturing Date: 21JUN2022

BEI Resources is committed to ensuring digital accessibility for people with disabilities. This Certificate of Analysis contains complex tables and may not be fully accessible. Please let us know if you encounter accessibility barriers and a fully accessible document will be provided: E-mail: Contact@BEIResources.org. We try to respond to feedback within 24 hours.

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 hCoV-19/South Africa/ CERI-KRISP-K040013/2022 (GISAID: EPI_ISL_12268493.2)	99.98% identity with hCoV-19/South Africa/ CERI-KRISP-K040013/2022 (GISAID: EPI_ISL_12268493.2)
Titer by TCID ₅₀ Assay in Calu-3 Cells by Cytopathic Effect ¹ (6 days at 37°C and 5% CO ₂)	Report results	4.8 × 10 ⁵ 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 (2.8 × 10⁵ per mL, 2.8 × 10⁵ per mL and 8.9 × 10⁵ 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

15 JAN 2023

Technical 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.

ATCC® is a trademark of the American Type Culture Collection.

You are authorized to use this product for research use only. It is not intended for human use.



APPENDIX I: NGS Information for NR-56798 lot 70053884

Sequence analysis using AMGP readsQC-illumina.py pipeline and variant caller LoFreq version: 2.1.5 resulted in the discovery of five SNPs when compared to the reference sequence from GISAID EPI_ISL_12268493.2 (see Table I below). Additionally, both the reference sequence EPI_ISL_12268493.2 and NR-56798 lot 70053884 contained sixty-eight SNPs and five deletions (DEL) when compared to GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) (see Table II below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Table I: Variants with different nucleotides between NR-56798 lot 70053884 and reference sequence EPI_ISL_12268493.2

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	c10116t	294	1	7.1429%	ORF1ab (nsp5)	T21I
SNP	a10512g	394	1	32.9949%	ORF1ab (nsp5)	D153G
SNP	t11463c	421	1	35.3919%	ORF1ab (nsp6)	M164T
SNP	t15028c	275	1	12.0000%	ORF1ab (nsp12)	Y530H
SNP	t19329g	273	1	33.3333%	ORF1ab (nsp14)	Silent mutation

Table II: Variants with different nucleotides between NR-56798 lot 70053884 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	t670g	N/A	1	100.0000%	ORF1ab (nsp1)	S135R
SNP	c1627t	N/A	1	100.0000%	ORF1ab (nsp2)	Silent mutation
SNP	c2790t	N/A	1	100.0000%	ORF1ab (nsp3)	T24I
SNP	c3037t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	g4184a	N/A	1	100.0000%	ORF1ab (nsp3)	G489S
SNP	c4321t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c9344t	N/A	1	100.0000%	ORF1ab (nsp4)	L264F
SNP	a9424g	N/A	1	100.0000%	ORF1ab (nsp4)	Silent mutation
SNP	c9534t	N/A	1	100.0000%	ORF1ab (nsp4)	T327I
SNP	c10029t	N/A	1	100.0000%	ORF1ab (nsp4)	T492I
SNP	c10198t	N/A	1	100.0000%	ORF1ab (nsp5)	Silent mutation
SNP	g10447a	N/A	1	100.0000%	ORF1ab (nsp5)	Silent mutation
SNP	c10449a	N/A	1	100.0000%	ORF1ab (nsp5)	P132H

BEI Resources

www.beiresources.org

E-mail: contact@beiresources.org

Tel: 800-359-7370

Fax: 703-365-2898

Certificate of Analysis for NR-56798

Variant Type	Variant Position and Identified Alternative Base	Coverage ¹	Length of Variant	Frequency of Variant ¹	Gene (Region)	Amino Acid Mutation
DEL	Δ11288-11296	N/A	-9	100.0000%	ORF1ab (nsp6)	ΔSGF (amino acids 106-108)
SNP	g12160a	N/A	1	100.0000%	ORF1ab (nsp8)	Silent mutation
SNP	g12310a	N/A	1	100.0000%	ORF1ab (nsp8)	Silent mutation
SNP	c12880t	N/A	1	100.0000%	ORF1ab (nsp9)	Silent mutation
SNP	c14408t	N/A	1	100.0000%	ORF1ab (nsp12)	P323L
SNP	c15714t	N/A	1	100.0000%	ORF1ab (nsp12)	Silent mutation
SNP	c17410t	N/A	1	100.0000%	ORF1ab (nsp13)	R392C
SNP	a18163g	N/A	1	100.0000%	ORF1ab (nsp14)	I42V
SNP	c19955t	N/A	1	100.0000%	ORF1ab (nsp15)	T112I
SNP	a20055g	N/A	1	100.0000%	ORF1ab (nsp15)	Silent mutation
SNP	c21618t	N/A	1	100.0000%	Spike	T19I
DEL	Δ21633-21641	N/A	-9	100.0000%	Spike	ΔLPP (amino acids 24-26), A27S
DEL	Δ21765-21770	N/A	-6	100.0000%	Spike	ΔHV (amino acids 69-70)
SNP	g21987a	N/A	1	100.0000%	Spike	G142D
SNP	t22200g	N/A	1	100.0000%	Spike	V213G
SNP	g22578a	N/A	1	100.0000%	Spike	G339D
SNP	c22674t	N/A	1	100.0000%	Spike	S371F
SNP	t22679c	N/A	1	100.0000%	Spike	S373P
SNP	c22686t	N/A	1	100.0000%	Spike	S375F
SNP	a22688g	N/A	1	100.0000%	Spike	T376A
SNP	g22775a	N/A	1	100.0000%	Spike	D405N
SNP	a22786c	N/A	1	100.0000%	Spike	R408S
SNP	g22813t	N/A	1	100.0000%	Spike	K417N
SNP	t22882g	N/A	1	100.0000%	Spike	N440K
SNP	t22917g	N/A	1	100.0000%	Spike	L452R
SNP	g22992a	N/A	1	100.0000%	Spike	S477N
SNP	c22995a	N/A	1	100.0000%	Spike	T478K
SNP	a23013c	N/A	1	100.0000%	Spike	E484A
SNP	t23018g	N/A	1	100.0000%	Spike	F486V
SNP	a23055g	N/A	1	100.0000%	Spike	Q498R
SNP	a23063t	N/A	1	100.0000%	Spike	N501Y
SNP	t23075c	N/A	1	100.0000%	Spike	Y505H
SNP	a23403g	N/A	1	100.0000%	Spike	D614G
SNP	c23525t	N/A	1	100.0000%	Spike	H655Y
SNP	t23599g	N/A	1	100.0000%	Spike	N679K
SNP	c23604a	N/A	1	100.0000%	Spike	P681H
SNP	c23854a	N/A	1	100.0000%	Spike	N764K
SNP	g23948t	N/A	1	100.0000%	Spike	D796Y

Certificate of Analysis for NR-56798

Variant Type	Variant Position and Identified Alternative Base	Coverage ¹	Length of Variant	Frequency of Variant ¹	Gene (Region)	Amino Acid Mutation
SNP	a24424t	N/A	1	100.0000%	Spike	Q954H
SNP	t24469a	N/A	1	100.0000%	Spike	N969K
SNP	c25000t	N/A	1	100.0000%	Spike	Silent mutation
SNP	c25584t	N/A	1	100.0000%	ORF3a	Silent mutation
SNP	c26060t	N/A	1	100.0000%	ORF3a	T223I
SNP	c26270t	N/A	1	100.0000%	Envelope	T9I
SNP	g26529a	N/A	1	100.0000%	Membrane	D3N
SNP	c26577g	N/A	1	100.0000%	Membrane	Q19E
SNP	g26709a	N/A	1	100.0000%	Membrane	A63T
SNP	c27807t	N/A	1	100.0000%	ORF7b	Silent mutation
SNP	c27889t	N/A	1	100.0000%	Intergenic (ORF7b/ORF8)	Untranslated
SNP	a28271t	N/A	1	100.0000%	Intergenic (ORF8/Nucleocapsid)	Untranslated
SNP	c28311t	N/A	1	100.0000%	Nucleocapsid	P13L
SNP	a28330g	N/A	1	100.0000%	Nucleocapsid	Silent mutation
DEL	Δ28362-28370	N/A	-9	100.0000%	Nucleocapsid	ΔERS (amino acids 31-33)
SNP	g28881a	N/A	1	100.0000%	Nucleocapsid	R203K
SNP	g28882a	N/A	1	100.0000%		
SNP	g28883c	N/A	1	100.0000%		
SNP	a29510c	N/A	1	100.0000%	Nucleocapsid	G204R
SNP	a29510c	N/A	1	100.0000%	Nucleocapsid	S413R
DEL	Δ29734-29759	N/A	-26	100.0000%	3'UTR	Untranslated
SNP	g29868a	N/A	1	100.0000%	3'UTR	Untranslated

¹Coverage for all variants in Table II 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 II are mismatches in 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.