

SARS-Related Coronavirus 2, Isolate hCoV-19/USA/MD-HP30386/2022 (Lineage BA.4; Omicron Variant)

Catalog No. NR-56803

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/USA/MD-HP30386/2022 was isolated from a human on April 9, 2022, in Maryland, USA. NR-56803 lot 70053069 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 7 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:

VTA(1)VT(1)/C(1) (Johns Hopkins University/BEI Resources); VTA = *Cercopithecus aethiops* kidney cells with transmembrane protease, serine 2 gene (Vero E6-TMPRSS2) and human angiotensin-converting enzyme 2; VT = *Cercopithecus aethiops* kidney cells with transmembrane protease, serine 2 gene (Vero E6-TMPRSS2); C = Calu-3

Lot: 70053069

Manufacturing Date: 03JUN2022

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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 SARS-CoV-2, hCoV-19/USA/MD-HP30386/2022 (GISAID: EPI_ISL_12416220)	99.99% identity with SARS-CoV-2, hCoV-19/USA/MD-HP30386/2022 (GISAID: EPI_ISL_12416220)
Titer by TCID₅₀ Assay in Calu-3 Cells by Cytopathic Effect¹ (7 days at 37°C and 5% CO ₂)	Report results	6.9 × 10 ³ 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 × 10³ per mL, 8.9 × 10³ per mL and 8.9 × 10³ 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.

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15 JAN 2023

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APPENDIX I: NGS Information for NR-56803 lot 70053069

Note: The sequence submitted to GISAID for this isolate (EPI_ISL_12416220) contains stretches of ambiguous nucleotides (Ns), which required selection of an alternate sequence to serve as a suitable reference for comparison of the sequence from NR-56803. The reference sequence used for quality control testing and reporting purposes is a modified sequence of EPI_ISL_12416220 in which the ambiguous Ns are replaced by the corresponding nucleotides from the Wuhan-Hu-1 sequence. Nine of the reported amino acid mutations occurred in the reference modified region.

Sequence analysis using AMGP readsQC-illumina.py pipeline and variant caller LoFreq version: 2.1.5 resulted in the discovery of thirteen SNPs when compared to the modified reference sequence from GISAID EPI_ISL_12416220 (see Table I below). Additionally, both the modified reference sequence EPI_ISL_12416220 and NR-56803 lot 70053069 contained sixty-two SNPs and five 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-56803 lot 70053069 and modified reference sequence EPI_ISL_12416220

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	t1963g	106	1	5.6604%	ORF1ab (nsp2)	Silent mutation
SNP	c12741t	249	1	99.5984%	ORF1ab (nsp9)	T19I
SNP	t14679c	136	1	8.0882%	ORF1ab (nsp12)	Silent mutation
SNP	c20956t	109	1	8.2569%	ORF1ab (nsp16)	L100F
SNP	t22882g	114	1	100.0000%	Spike	N440K ¹
SNP	t22917g	107	1	100.0000%	Spike	L452R ¹
SNP	g22992a	181	1	100.0000%	Spike	S477N ¹
SNP	c22995a	183	1	100.0000%	Spike	T478K ¹
SNP	a23013c	185	1	98.9189%	Spike	E484A ¹
SNP	t23018g	193	1	98.9637%	Spike	F486V ¹
SNP	a23055g	198	1	100.0000%	Spike	Q498R ¹
SNP	a23063t	197	1	100.0000%	Spike	N501Y ¹
SNP	t23075c	203	1	100.0000%	Spike	Y505H ¹

¹Amino acid mutation is in a reference modified region.

Table II: Variants with different nucleotides between NR-56803 lot 70053069 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	t670g	N/A	1	100.0000%	ORF1ab (nsp1)	S135R
DEL	686-694	N/A	-9	100.0000%	ORF1ab (nsp1)	ΔKSF (amino acids 141-143)
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	c6723t	N/A	1	100.0000%	ORF1ab (nsp3)	T1335I
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
DEL	11288-11296	N/A	-9	100.0000%	ORF1ab (nsp6)	ΔSGF (amino acids 106-108)
SNP	g12160a	N/A	1	100.0000%	ORF1ab (nsp8)	Silent mutation
SNP	c12439t	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	t21570g	N/A	1	100.0000%	Spike	V3G
SNP	c21618t	N/A	1	100.0000%	Spike	T19I
DEL	21633-21641	N/A	-9	100.0000%	Spike	ΔLPP (amino acids 24-26), A27S
DEL	21765-21770	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

Variant Type	Variant Position and Identified Alternative Base	Coverage ¹	Length of Variant	Frequency of Variant ¹	Gene (Region)	Amino Acid Mutation
SNP	a22786c	N/A	1	100.0000%	Spike	R408S
SNP	g22813t	N/A	1	100.0000%	Spike	K417N
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	P618H
SNP	c23854a	N/A	1	100.0000%	Spike	N764K
SNP	g23948t	N/A	1	100.0000%	Spike	D796Y
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	c26577g	N/A	1	100.0000%	Membrane	Q19E
SNP	g26709a	N/A	1	100.0000%	Membrane	A63T
SNP	c26858t	N/A	1	100.0000%	Membrane	Silent mutation
SNP	a27259c	N/A	1	100.0000%	ORF6	Silent mutation
SNP	g27382c	N/A	1	100.0000%	ORF6	D61L
SNP	a27383t	N/A	1	100.0000%		
SNP	t27384c	N/A	1	100.0000%		
SNP	g27788t	N/A	1	100.0000%	ORF7b	L11F
SNP	c27807t	N/A	1	100.0000%	ORF7b	Silent mutation
SNP	a28271t	N/A	1	100.0000%	Intergenic – ORF8/Nucleotide	Untranslated
SNP	c28311t	N/A	1	100.0000%	Nucleotide	P13L
DEL	28362-28370	N/A	-9	100.0000%	Nucleotide	ΔERS (amino acids 31-33)
SNP	c28724t	N/A	1	100.0000%	Nucleotide	P151S
SNP	g28881a	N/A	1	100.0000%	Nucleotide	R203K
SNP	g28882a	N/A	1	100.0000%		
SNP	g28883c	N/A	1	100.0000%	Nucleotide	G204R
SNP	a29510c	N/A	1	100.0000%	Nucleotide	S413R

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.