

SARS-Related Coronavirus 2, Isolate hCoV-19/USA/VA-FBCH_675/2021 (Lineage AY4.2; Delta Variant)

Catalog No. NR-56519

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/USA/VA-FBCH_675/2021 was isolated from a human on September 17, 2021, in Virginia, USA. NR-56519 lot 70050558 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(2)/C(1) (Centers for Disease Control and Prevention/BEI Resources); VT = *Cercopithecus aethiops* kidney cells with transmembrane protease, serine 2 gene (TMPRSS2); C = Calu-3

Lot: 70050558

Manufacturing Date: 10FEB2022

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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/VA-FBCH_675/2021 (GISAID: EPI_ISL_5852425)	99.99% identity with SARS-CoV-2, hCoV-19/USA/VA-FBCH_675/2021 (GISAID: EPI_ISL_5852425)
Titer by TCID₅₀ Assay in Calu-3 Cells by Cytopathic Effect¹ (7 days at 37°C and 5% CO ₂)	Report results	4.4 × 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, 2.8 × 10⁶ per mL and 8.9 × 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.

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APPENDIX I: NGS Information for NR-56519 lot 70050558

Sequence analysis using AMGP readsQC-illumina.py pipeline and variant caller LoFreq version: 2.1.5 resulted in the discovery of two SNPs when compared to the reference sequence from GISAID EPI_ISL_5852425 (see Table I below). Additionally, both the reference sequence EPI_ISL_5852425 and NR-56519 lot 70050558 contained forty-five SNPs and three 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-56519 lot 70050558 and reference sequence EPI_ISL_5852425

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	t14679c	1466	1	6.2074%	ORF1ab (nsp12)	Silent mutation
SNP	t22114c	1027	1	6.2317%	Spike	Silent mutation

Table II: Variants with different nucleotides between NR-56519 lot 70050558 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	g210t	N/A	1	100.0000%	5'UTR	Untranslated
SNP	c241t	N/A	1	100.0000%	5'UTR	Untranslated
SNP	c2110t	N/A	1	100.0000%	ORF1ab (nsp2)	Silent mutation
SNP	c3037t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	g4181t	N/A	1	100.0000%	ORF1ab (nsp3)	A488S
SNP	c4423t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c6401t	N/A	1	100.0000%	ORF1ab (nsp3)	P1228L
SNP	c6402t	N/A	1	100.0000%		P1469S
SNP	c7124t	N/A	1	100.0000%	ORF1ab (nsp3)	A1711V
SNP	c7851t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c8986t	N/A	1	100.0000%	ORF1ab (nsp4)	V167L
SNP	g9053t	N/A	1	100.0000%	ORF1ab (nsp4)	T492I
SNP	c10029t	N/A	1	100.0000%	ORF1ab (nsp4)	T77A
SNP	a11201g	N/A	1	100.0000%	ORF1ab (nsp6)	Silent mutation
SNP	a11332g	N/A	1	100.0000%	ORF1ab (nsp6)	P323L
SNP	c14408t	N/A	1	100.0000%	ORF1ab (nsp12)	Silent mutation
SNP	t14679c	N/A	1	100.0000%	ORF1ab (nsp12)	Silent mutation

Variant Type	Variant Position and Identified Alternative Base	Coverage ¹	Length of Variant	Frequency of Variant ¹	Gene (Region)	Amino Acid Mutation
SNP	g15451a	N/A	1	100.0000%	ORF1ab (nsp12)	G671S
SNP	c16466t	N/A	1	100.0000%	ORF1ab (nsp13)	P77L
SNP	t17040c	N/A	1	100.0000%	ORF1ab (nsp13)	Silent mutation
SNP	c19220t	N/A	1	100.0000%	ORF1ab (nsp14)	A394V
SNP	c21618g	N/A	1	100.0000%	Spike	T19R
SNP	c21846t	N/A	1	100.0000%	Spike	T95I
SNP	g21987a	N/A	1	100.0000%	Spike	G142D
SNP	t21995c	N/A	1	100.0000%	Spike	Y145H
SNP	g22017t	N/A	1	100.0000%	Spike	W152L
DEL	Δ22029-22034	N/A	-6	100.0000%	Spike	E156G, ΔFR (amino acids 157-158)
SNP	c22227t	N/A	1	100.0000%	Spike	A222V
SNP	g22487c	N/A	1	100.0000%	Spike	E309Q
SNP	t22917g	N/A	1	100.0000%	Spike	L452R
SNP	c22995a	N/A	1	100.0000%	Spike	T478K
SNP	a23403g	N/A	1	100.0000%	Spike	D614G
SNP	c23604g	N/A	1	100.0000%	Spike	P681R
SNP	g24410a	N/A	1	100.0000%	Spike	D950N
SNP	c25469t	N/A	1	100.0000%	ORF3a	S26L
SNP	c25614t	N/A	1	100.0000%	ORF3a	Silent mutation
SNP	g26526t	N/A	1	100.0000%	Membrane	A2S
SNP	t26767c	N/A	1	100.0000%	Membrane	I82T
SNP	t27638c	N/A	1	100.0000%	ORF7a	V82A
SNP	c27752t	N/A	1	100.0000%	ORF7a	T120I
SNP	c27874t	N/A	1	100.0000%	ORF7b	T40I
DEL	Δ28248-28253	N/A	-6	100.0000%	ORF8	ΔDF (amino acids 119-120)
DEL	Δ28271	N/A	1	100.0000%	Intergenic (ORF8/ Nucleocapsid)	Untranslated
SNP	a28461g	N/A	1	100.0000%	Nucleocapsid	D63G
SNP	g28881t	N/A	1	100.0000%	Nucleocapsid	R203M
SNP	g28916t	N/A	1	100.0000%	Nucleocapsid	G215C
SNP	g29402t	N/A	1	100.0000%	Nucleocapsid	D377Y
SNP	g29742t	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.