

**SARS-Related Coronavirus 2, Isolate hCoV-19/USA/CA-VRLC086/2021 (Delta Variant)**

**Catalog No. NR-55691**

**Product Description:**

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/USA/CA-VRLC086/2021 was isolated from a human mid-turbinate nasal swab in California, USA on June 21, 2021. NR-55691 lot 70046650 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% CO<sub>2</sub>. The cells and supernatant were spin-clarified at 1500 × g for 10 minutes at 4°C.

**Passage History:**

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

**Lot: 70046650**

**Manufacturing Date: 09AUG2021**

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TEST	SPECIFICATIONS	RESULTS
<b>Identification by Infectivity in Calu-3 Cells</b>	Cell rounding and detachment	Cell rounding and detachment
<b>Next-Generation Sequencing (NGS) of Complete Genome Using Illumina® iSeq™ 100 Platform</b> (Refer to Appendix I for NGS information)	≥ 98% identity with SARS-CoV-2, hCoV-19/USA/CA-VRLC086/2021 (GISAID: EPI_ISL_2987140)	99.8% identity with SARS-CoV-2, hCoV-19/USA/CA-VRLC086/2021 (GISAID: EPI_ISL_2987140)
<b>Titer by TCID<sub>50</sub> Assay in Calu-3 Cells by Cytopathic Effect<sup>1</sup></b> (6 days at 37°C and 5% CO <sub>2</sub> )	Report results	4.8 × 10 <sup>6</sup> TCID <sub>50</sub> per mL <sup>2</sup>
<b>Sterility (21-day incubation)</b> Harpo's HTYE broth, 37°C and 26°C, aerobic <sup>3</sup> 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
<b>Mycoplasma Contamination</b> 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

<sup>1</sup>The Tissue Culture Infectious Dose 50% (TCID<sub>50</sub>) endpoint is the 50% infectious endpoint in cell culture. The TCID<sub>50</sub> 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% (LD<sub>50</sub>) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID<sub>50</sub> provides a measure of the titer (or infectivity) of a virus preparation.

<sup>2</sup>Titer was determined by cytopathic effects (CPE) and completed in triplicate (8.9 × 10<sup>6</sup> per mL, 2.8 × 10<sup>6</sup> per mL and 2.8 × 10<sup>6</sup> per mL). The average of the three values is reported.

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

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Program Manager or designee, ATCC Federal Solutions

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**APPENDIX I: NGS Information for NR-55691 lot 70046650**

Sequence analysis using SBC v2.0 pipeline and freebayes v1.3.1 variant caller resulted in the discovery of two SNPs when compared to the reference sequence EPI\_ISL\_2987140 (see Table I below). Additionally, both the reference sequence EPI\_ISL\_2987140 and NR-55691 lot 70046650 contained thirty-seven SNPs and three 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-55691 lot 70046650 and reference sequence EPI\_ISL\_2987140**

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	c6402t	6069	1	99.9176%	ORF1ab (nsp3)	P1228L
SNP	c19220t	3968	1	99.6220%	ORF1ab (nsp14)	A394V

**Table II: Variants with different nucleotides between NR-55691 lot 70046650 and GenBank MN908947.3 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome)**

Variant Type	Variant Position and Identified Alternative Base	Coverage <sup>1</sup>	Length of Variant	Frequency of Variant <sup>1</sup>	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	g2272c	N/A	1	100.0000%	ORF1ab (nsp2)	K489N
SNP	c3037t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	g4181t	N/A	1	100.0000%	ORF1ab (nsp3)	A488S
SNP	c7124t	N/A	1	100.0000%	ORF1ab (nsp3)	P1469S
SNP	a8062g	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c8986t	N/A	1	100.0000%	ORF1ab (nsp4)	Silent mutation
SNP	g9053t	N/A	1	100.0000%	ORF1ab (nsp4)	V167L
SNP	c10029t	N/A	1	100.0000%	ORF1ab (nsp4)	T492I
SNP	a11201g	N/A	1	100.0000%	ORF1ab (nsp6)	T77A
SNP	a11332g	N/A	1	100.0000%	ORF1ab (nsp6)	Silent mutation
SNP	c14408t	N/A	1	100.0000%	ORF1ab (nsp12)	P323L
SNP	g15451a	N/A	1	100.0000%	ORF1ab (nsp12)	G671S
SNP	c16466t	N/A	1	100.0000%	ORF1ab (nsp13)	P77L
SNP	c20436t	N/A	1	100.0000%	ORF1ab (nsp15)	Silent mutation
SNP	c21618g	N/A	1	100.0000%	Spike	T19R

Variant Type	Variant Position and Identified Alternative Base	Coverage <sup>1</sup>	Length of Variant	Frequency of Variant <sup>1</sup>	Gene (Region)	Amino Acid Mutation
SNP	c21846t	N/A	1	100.0000%	Spike	T95I
SNP	g21987a	N/A	1	100.0000%	Spike	G142D
DEL	Δ22029-22034	N/A	-6	100.0000%	Spike	E156G, ΔFR (amino acids 157-158)
SNP	g22335t	N/A	1	100.0000%	Spike	W258L
SNP	g22813t	N/A	1	100.0000%	Spike	K417N
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	c25549t	N/A	1	100.0000%	ORF3a	L53F
SNP	c26607t	N/A	1	100.0000%	Membrane protein	L29F
SNP	t26767c	N/A	1	100.0000%	Membrane protein	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

<sup>1</sup>Coverage 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.