

**SARS-Related Coronavirus 2, Isolate hCoV-19/USA/CA-Stanford-15\_S02/2021 (Lineage B.1.617.1; Kappa Variant)**

**Catalog No. NR-55486**

**Product Description:**

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/USA/CA-Stanford-15\_S02/2021 was isolated from a mid-turbinate nasal swab from a 29-year-old male in California, USA on March 5, 2021. NR-55486 lot 70044706 was produced by infecting *Cercopithecus aethiops* kidney epithelial cells with transmembrane protease, serine 2 gene (Vero E6-TMPRSS2) 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(3)/VT(1) (Prior to deposit at BEI Resources/BEI Resources); VT = *Cercopithecus aethiops* kidney cells with transmembrane protease, serine 2 gene (Vero E6-TMPRSS2)

**Lot: 70044706**

**Manufacturing Date: 17MAY2021**

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TEST	SPECIFICATIONS	RESULTS
<b>Identification by Infectivity in Vero E6-TMPRSS2 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-Stanford-15_S02/2021 (GISAID: EPI_ISL_1675223)	99.96% identity with SARS-CoV-2, hCoV-19/USA/CA-Stanford-15_S02/2021 (GISAID: EPI_ISL_1675223)
<b>Titer by TCID<sub>50</sub> Assay in Vero E6-TMPRSS2 Cells by Cytopathic Effect<sup>1</sup></b> (5 days at 37°C and 5% CO <sub>2</sub> )	Report results	1.8 × 10 <sup>7</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 (2.8 × 10<sup>7</sup> per mL, 8.9 × 10<sup>6</sup> per mL and 1.6 × 10<sup>7</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.

/Heather Couch/  
Heather Couch

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Program Manager or designee, ATCC Federal Solutions

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**APPENDIX I: NGS Information for NR-55486 lot 70044706**

Sequence analysis using SBC v2.0 pipeline and freebayes v1.3.1 variant caller resulted in the discovery of six SNPs when compared to the reference sequence from EPI\_ISL\_1675223 (see Table I below). Additionally, both the reference sequence EPI\_ISL\_1675223 and NR-55486 lot 70044706 contained twenty-five SNPs and one deletion (DEL) when compared to GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) (see Table II below).

**Table I: Variants with different nucleotides between NR-55486 lot 70044706 and reference sequence EPI\_ISL\_1675223**

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	t14679c	1699	1	6.9453%	ORF1ab (nsp12)	Silent mutation
SNP	c16362t	2462	1	99.2689%	ORF1ab (nsp13)	Silent mutation
SNP	c18511t	3951	1	99.8988%	ORF1ab (nsp14)	P158S
SNP	c20016t	5589	1	99.4274%	ORF1ab (nsp15)	Silent mutation
SNP	c26256a	3568	1	99.9720%	Envelope	F4L
SNP	t29760c_rev_t <sup>1</sup>	4260	1	99.9296%	3'UTR	Untranslated region

<sup>1</sup>t29760c represents the original nucleotide of 't' in GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) to nucleotide 'c' in reference sequence GISAID EPI\_ISL\_1675223. \_rev\_t represents the nucleotide 't' observed in NR-55486 lot 70044706.

**Table II: Variants with different nucleotides between NR-55486 lot 70044706 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 region
SNP	c241t	N/A	1	100.0000%	5'UTR	Untranslated region
SNP	c3037t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c3457t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c4965t	N/A	1	100.0000%	ORF1ab (nsp3)	T749I
SNP	a11201g	N/A	1	100.0000%	ORF1ab (nsp6)	T77A
SNP	c14408t	N/A	1	100.0000%	ORF1ab (nsp12)	P323L
SNP	g17523t	N/A	1	100.0000%	ORF1ab (nsp13)	M429I
SNP	a20396g	N/A	1	100.0000%	ORF1ab (nsp15)	K259R
SNP	c20936t	N/A	1	100.0000%	ORF1ab (nsp16)	T93M
SNP	t21895c	N/A	1	100.0000%	Spike	Silent mutation
SNP	g21987a	N/A	1	100.0000%	Spike	G142D

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	g22022a	N/A	1	100.0000%	Spike	E154K
SNP	t22917g	N/A	1	100.0000%	Spike	L452R
SNP	g23012c	N/A	1	100.0000%	Spike	E484Q
SNP	a23403g	N/A	1	100.0000%	Spike	D614G
SNP	c23604g	N/A	1	100.0000%	Spike	P681R
SNP	a24775t	N/A	1	100.0000%	Spike	Q1071H
SNP	c24863g	N/A	1	100.0000%	Spike	H1101D
SNP	c25276t	N/A	1	100.0000%	Spike	Silent mutation
SNP	c25469t	N/A	1	100.0000%	ORF3a	S26L
SNP	t27299c	N/A	1	100.0000%	ORF6	I33T
SNP	t27638c	N/A	1	100.0000%	ORF7a	V82A
DEL	Δ28271	N/A	-1	100.0000%	Intergenic (ORF8/Nucleocapsid)	Untranslated region
SNP	g28881t	N/A	1	100.0000%	Nucleocapsid	R203M
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 (EPI\_ISL\_1675223) 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.