

SARS-Related Coronavirus 2, Isolate hCoV-19/USA/CA-Stanford-15_S02/2021 (Lineage B.1.617.1; Kappa Variant) in Calu-3 Cells

Catalog No. NR-55472

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-55472 lot 70044632 was produced by infecting *Homo sapiens* lung adenocarcinoma 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(3)/C(1) (Stanford University/BEI Resources); VT = *Cercopithecus aethiops* kidney cells with transmembrane protease, serine 2 gene (Vero E6-TMPRSS2); C = Calu-3 cells

Lot: 70044632

Manufacturing Date: 10MAY2021

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 SARS-CoV-2, hCoV-19/USA/CA-Stanford-15_S02/2021 (GISAID: EPI_ISL_1675223)	99.95% identity with SARS-CoV-2, hCoV-19/USA/CA-Stanford-15_S02/2021 (GISAID: EPI_ISL_1675223)
Titer by TCID₅₀ Assay in Calu-3 Cells by Cytopathic Effect¹ (6 days at 37°C and 5% CO ₂)	Report results	3.8 × 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 (1.6 × 10⁵ per mL in 5 days, 8.9 × 10⁴ per mL in 6 days and 8.9 × 10⁵ per mL in 6 days). 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.

/Heather Couch/

Heather Couch

01 JUL 2021

Program 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-55472 lot 70044632

Sequence analysis using SBC v2.0 pipeline and freebayes v1.3.1 variant caller resulted in the discovery of ten 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-55472 lot 70044632 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-55472 lot 70044632 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	1761	1	13.7990%	ORF1ab (nsp12)	Silent mutation
SNP	t15909c	2021	1	5.7397%	ORF1ab (nsp12)	Silent mutation
SNP	c16362t	2009	1	99.6018%	ORF1ab (nsp13)	Silent mutation
SNP	t17886c	2061	1	5.3857%	ORF1ab (nsp13)	Silent mutation
SNP	c18511t	3773	1	99.5494%	ORF1ab (nsp14)	P158S
SNP	c20016t	5086	1	99.5871%	ORF1ab (nsp15)	Silent mutation
SNP	t22114c	759	1	9.3544%	Spike	Silent mutation
SNP	c26256a	3456	1	99.8843%	Envelope protein	F4L
SNP	g29742t	3670	1	99.8910%	3'UTR	Untranslated region
SNP	t29760c_rev_t ¹	3485	1	99.7991%	3'UTR	Untranslated region

¹t29760c 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-55472 lot 70044632.

Table II: Variants with different nucleotides between NR-55472 lot 70044632 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 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

Variant Type	Variant Position and Identified Alternative Base	Coverage ¹	Length of Variant	Frequency of Variant ¹	Gene (Region)	Amino Acid Mutation
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
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 protein	R203M
SNP	g29402t	N/A	1	100.0000%	Nucleocapsid protein	D377Y

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 (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.