Certificate of Analysis for NR-59441

SARS-Related Coronavirus 2, Isolate hCoV-19/USA/CA-Stanford-139_S35/2023 (Lineage XBB.1.9)

Catalog No. NR-59441

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/USA/CA-Stanford-139_S35/2023 was isolated from a human nasopharyngeal swab on March 28, 2023, in California, USA. NR-59441 lot 70060986 was produced by infecting *Chlorocebus* (formerly *Cercopithecus*) *aethiops (C. aethiops)* kidney epithelial cells expressing transmembrane protease, serine 2 and human angiotensin-converting enzyme 2 (Vero E6-TMPRSS2-T2A-ACE2; VTA; BEI Resources NR-54970) with the deposited material and incubating in Eagle's Minimum Essential Medium containing Earle's Balanced Salt Solution, non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate and 1.5 g/L of sodium bicarbonate (ATCC® 30-2003™), supplemented with 2% fetal bovine serum (ATCC® 30-2020™) for 3 days at 37°C with 5% CO_2. The cells and supernatant were spin-clarified at 1500 × g for 10 minutes at 4°C.

Passage History:

VT(5, including plaque purification)/VTA(1) (Emory University/BEI Resources); VT = *C. aethiops* kidney cells expressing transmembrane protease, serine 2 gene (Vero E6-TMPRSS2); VTA = *C. aethiops* kidney cells expressing transmembrane protease, serine 2 gene and human angiotensin-converting enzyme 2 (Vero E6-TMPRSS2-T2A-ACE2)

Lot: 70060986 Manufacturing Date: 31JUL2023

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in VTA Cells	Cell rounding and detachment	Cell rounding and detachment
Next-Generation Sequencing (NGS) of Complete Genome Using Illumina® MiSeq™ 100 Platform (Refer to Appendix I for NGS information)	≥ 98% identity with isolate hCoV-19/USA/CA-Stanford-139_S35/2023 (GISAID: EPI_ISL_17417339)	99.93% identity with isolate hCoV-19/USA/CA-Stanford-139_S35/2023 (GISAID: EPI_ISL_17417339)
Titer by $TCID_{50}$ Assay in VTA Cells by Cytopathic Effect[1,2] (6 days at 37°C and 5% CO_2)	Report results	7.2 × 10^6 $TCID_{50}$ per mL
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic[3]	No growth	Pending
Trypticase Soy broth, 37°C and 26°C, aerobic	No growth	Pending
Sabouraud broth, 37°C and 26°C, aerobic	No growth	Pending
Blood agar, 37°C, aerobic	No growth	Pending
Blood agar, 37°C, anaerobic	No growth	Pending
Thioglycollate broth, 37°C, anaerobic	No growth	Pending
DMEM with 10% FBS, 37°C and 5% CO_2	No growth	Pending
Mycoplasma Contamination		
Agar and broth culture (14-day incubation at 37°C)	None detected	Pending
DNA detection by PCR of extracted Test Article nucleic acid	None detected	Pending

[1]The Tissue Culture Infectious Dose 50% ($TCID_{50}$) endpoint is the 50% infectious endpoint in cell culture. The $TCID_{50}$ 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_{50}) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the $TCID_{50}$ provides a measure of the titer (or infectivity) of a virus preparation.
[2]Titer was determined by cytopathic effects (CPE) and completed in triplicate (2.8 × 10^6 per mL, 2.8 × 10^6 per mL and 1.6 × 10^7 per mL). The average of the three values is reported.
[3]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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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.

Support Provided by NIAID

APPENDIX I: NGS Information for NR-59441 lot 70060986

Sequence analysis using AMGP readsQC-illumina.py pipeline and variant caller LoFreq version: 2.1.5 resulted in the discovery of two SNPs and two DEL when compared to GISAID EPI_ISL_17417339 (see Table I below). Additionally, both the reference sequence EPI_ISL_17417339 and NR-59441 lot 70060986 contained one hundred four SNPs and four 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-59441 lot 70060986 and reference sequence EPI_ISL_17417339

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	t3256g	3667	1	6.4903%	ORF1ab (nsp3)	N179K
DEL	Δ26284-26286	N/A	-3	100.0000%	Envelope	ΔV14
SNP	t27479c	3988	1	6.4193%	ORF7a	V29A
DEL	Δ28362-28370	N/A	-9	100.0000%	Nucleocapsid	ΔERS (amino acids 31-33)

Table II: Variants with different nucleotides between NR-59441 lot 70060986 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	c203t	N/A	1	100.0000%	5'UTR	Untranslated
SNP	c241t	N/A	1	100.0000%	5'UTR	Untranslated
SNP	a405g	N/A	1	100.0000%	ORF1ab (nsp1)	K47R
SNP	t670g	N/A	1	100.0000%	ORF1ab (nsp1)	S135R
SNP	c2450t	N/A	1	100.0000%	ORF1ab (nsp2)	Silent mutation
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	t4579a	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	g5720a	N/A	1	100.0000%	ORF1ab (nsp3)	G1001S
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

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Variant Type	Variant Position and Identified Alternative Base	Coverage[1]	Length of Variant	Frequency of Variant[1]	Gene (Region)	Amino Acid Mutation
SNP	c9866t	N/A	1	100.0000%	ORF1ab (nsp4)	L438F
SNP	a9925t	N/A	1	100.0000%	ORF1ab (nsp4)	Silent mutation
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
SNP	c10714t	N/A	1	100.0000%	ORF1ab (nsp5)	Silent mutation
DEL	Δ11288-11296	N/A	-9	100.0000%	ORF1ab (nsp6)	ΔSGF (amino acids 106-108)
SNP	t11619c	N/A	1	100.0000%	ORF1ab (nsp6)	F216S
SNP	c11956t	N/A	1	100.0000%	ORF1ab (nsp7)	Silent mutation
SNP	c12789t	N/A	1	100.0000%	ORF1ab (nsp9)	T35I
SNP	c12880t	N/A	1	100.0000%	ORF1ab (nsp9)	Silent mutation
SNP	c14408t	N/A	1	100.0000%	ORF1ab (nsp12)	P323L
SNP	a14850g	N/A	1	100.0000%	ORF1ab (nsp12)	Silent mutation
SNP	g15451a	N/A	1	100.0000%	ORF1ab (nsp12)	G671S
SNP	c15714t	N/A	1	100.0000%	ORF1ab (nsp12)	Silent mutation
SNP	c15738t	N/A	1	100.0000%	ORF1ab (nsp12)	Silent mutation
SNP	t15939c	N/A	1	100.0000%	ORF1ab (nsp12)	Silent mutation
SNP	t16342c	N/A	1	100.0000%	ORF1ab (nsp13)	S36P
SNP	c17410t	N/A	1	100.0000%	ORF1ab (nsp13)	R392C
SNP	c17747t	N/A	1	100.0000%	ORF1ab (nsp13)	P504L
SNP	t17859c	N/A	1	100.0000%	ORF1ab (nsp13)	Silent mutation
SNP	a18163g	N/A	1	100.0000%	ORF1ab (nsp14)	I42V
SNP	g18983a	N/A	1	100.0000%	ORF1ab (nsp14)	M315T
SNP	a19326g	N/A	1	100.0000%	ORF1ab (nsp14)	Silent mutation
SNP	c19955t	N/A	1	100.0000%	ORF1ab (nsp15)	T112I
SNP	a20055g	N/A	1	100.0000%	ORF1ab (nsp15)	Silent mutation
SNP	c21618t	N/A	1	100.0000%	Spike	T19I
DEL	Δ21633-21641	N/A	-9	100.0000%	Spike	A27S ΔLPP (amino acids 24-26)
SNP	t21810c	N/A	1	100.0000%	Spike	V83A
SNP	g21987a	N/A	1	100.0000%	Spike	G142D
DEL	Δ21991-21993	N/A	-3	100.0000%	Spike	ΔY144
SNP	c22000a	N/A	1	100.0000%	Spike	H146Q
SNP	c22109g	N/A	1	100.0000%	Spike	Q183E
SNP	t22200a	N/A	1	100.0000%	Spike	V213E
SNP	g22317t	N/A	1	100.0000%	Spike	G252V
SNP	g22577c	N/A	1	100.0000%	Spike	G339H

Variant Type	Variant Position and Identified Alternative Base	Coverage[1]	Length of Variant	Frequency of Variant[1]	Gene (Region)	Amino Acid Mutation
SNP	g22578a	N/A	1	100.0000%		
SNP	g22592t	N/A	1	100.0000%	Spike	A344S
SNP	g22599c	N/A	1	100.0000%	Spike	R346T
SNP	c22664a	N/A	1	100.0000%	Spike	L368I
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
SNP	a22786c	N/A	1	100.0000%	Spike	R408S
SNP	g22813t	N/A	1	100.0000%	Spike	K417N
SNP	t22882g	N/A	1	100.0000%	Spike	N440K
SNP	g22895c	N/A	1	100.0000%	Spike	V445P
SNP	t22896c	N/A	1	100.0000%		
SNP	g22898a	N/A	1	100.0000%	Spike	G446S
SNP	t22942g	N/A	1	100.0000%	Spike	N460K
SNP	g22992a	N/A	1	100.0000%	Spike	S477N
SNP	c22995a	N/A	1	100.0000%	Spike	T478K
SNP	a23013c	N/A	1	100.0000%	Spike	E484A
SNP	t23018c	N/A	1	100.0000%	Spike	F486P
SNP	t23019c	N/A	1	100.0000%		
SNP	t23031c	N/A	1	100.0000%	Spike	F490S
SNP	a23055g	N/A	1	100.0000%	Spike	Q498R
SNP	a23063t	N/A	1	100.0000%	Spike	N501Y
SNP	t23075c	N/A	1	100.0000%	Spike	Y505H
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	P681H
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	c25416t	N/A	1	100.0000%	ORF3a	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	a26275g	N/A	1	100.0000%	Envelope	T11A

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Variant Type	Variant Position and Identified Alternative Base	Coverage[1]	Length of Variant	Frequency of Variant[1]	Gene (Region)	Amino Acid Mutation
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	c27807t	N/A	1	100.0000%	ORF7b	Silent mutation
SNP	c27893t	N/A	1	100.0000%	Intergenic (ORF7b/ORF8)	Untranslated
SNP	g27915t	N/A	1	100.0000%	ORF8	G8stop
SNP	a28271t	N/A	1	100.0000%	Intergenic (ORF8/ Nucleocapsid)	Untranslated
SNP	t28297c	N/A	1	100.0000%	Nucleocapsid	Silent mutation
SNP	c28311t	N/A	1	100.0000%	Nucleocapsid	P13L
SNP	g28881a	N/A	1	100.0000%	Nucleocapsid	R203K
SNP	g28882a	N/A	1	100.0000%		
SNP	g28883c	N/A	1	100.0000%	Nucleocapsid	G204R
SNP	a29510c	N/A	1	100.0000%	Nucleocapsid	S413R
DEL	Δ29902	N/A	-1	100.0000%	3'UTR	Untranslated

[1]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.