Certificate of Analysis for NR-59786

SARS-Related Coronavirus 2, Isolate hCoV-19/USA/MD-HP50212/2023 (Lineage BA.2.86.1)

Catalog No. NR-59786

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/USA/MD-HP50212/2023 was isolated from a human in Maryland, USA, in 2023. NR-59786 lot 70067943 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(1)/VTA(1) (Johns Hopkins University/BEI Resources); VT = *C. aethiops* kidney cells expressing transmembrane protease, serine 2 gene (TMPRSS-2); VTA = *C. aethiops* kidney cells expressing TMPRSS-2 gene and human angiotensin-converting enzyme 2 (Vero E6-TMPRSS2-T2A-ACE2)

Lot: 70067943 **Manufacturing Date: 15APR2024**

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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™ Platform (Refer to Appendix I for NGS information)	≥ 98% identity with isolate hCoV-19/USA/MD-HP50212/2023 (GISAID: EPI_ISL_18679015)	99.96% identity with isolate hCoV-19/USA/MD-HP50212/2023 (GISAID: EPI_ISL_18679015)
Titer by $TCID_{50}$ Assay in VTA Cells by Cytopathic Effect[1,2] (6 days at 37°C and 5% CO_2)	Report results	2.4 × 10^6 $TCID_{50}$/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 (1.6 × 10^6 per mL, 2.8 × 10^6 per mL and 2.8 × 10^6 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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Technical 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.

Support Provided by NIAID

APPENDIX I: NGS Information for NR-59786 lot 70067943

Sequence analysis using AMGP readsQC-illumina.py pipeline and variant caller LoFreq version: 2.1.5 resulted in the discovery of one SNP when compared to reference sequence GISAID EPI_ISL_18679015 (see Table I below). Additionally, both the reference sequence GISAID EPI_ISL_18679015 and NR-59786 lot 70067943 contained eighty-one SNPs, six DELs and one INS 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-59786 lot 70067943 and reference sequence GISAID EPI_ISL_18679015

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	c11750t	1523	1	82.8628%	ORF1ab (nsp6)	L260F

Table II: Variants with different nucleotides between NR-59786 lot 70067943 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	c241t	N/A	1	100.0000%	5'UTR	Untranslated
SNP	t670g	N/A	1	100.0000%	ORF1ab (nsp1)	S135R
SNP	g677a	N/A	1	100.0000%	ORF1ab (nsp1)	A138T
SNP	c897a	N/A	1	100.0000%	ORF1ab (nsp2)	A31D
SNP	c2790t	N/A	1	100.0000%	ORF1ab (nsp3)	T24I
SNP	c3037t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	g3431t	N/A	1	100.0000%	ORF1ab (nsp3)	V238L
SNP	g4184a	N/A	1	100.0000%	ORF1ab (nsp3)	G489S
SNP	c4321t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	a6183g	N/A	1	100.0000%	ORF1ab (nsp3)	K1155R
SNP	a7842g	N/A	1	100.0000%	ORF1ab (nsp3)	N1708S
SNP	c8293t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	g8393a	N/A	1	100.0000%	ORF1ab (nsp3)	A1892T
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
SNP	c10029t	N/A	1	100.0000%	ORF1ab (nsp4)	T492I

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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	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	g11042t	N/A	1	100.0000%	ORF1ab (nsp6)	V24F
DEL	Δ11288-11296	N/A	-9	100.0000%	ORF1ab (nsp6)	ΔSGF (amino acids 106-108)
SNP	c12789t	N/A	1	100.0000%	ORF1ab (nsp9)	T35I
SNP	c12815t	N/A	1	100.0000%	ORF1ab (nsp9)	Silent mutation
SNP	c12880t	N/A	1	100.0000%	ORF1ab (nsp9)	Silent mutation
SNP	t13339c	N/A	1	100.0000%	ORF1ab (nsp10)	Silent mutation
SNP	c14408t	N/A	1	100.0000%	ORF1ab (nsp12)	P323L
SNP	c15714t	N/A	1	100.0000%	ORF1ab (nsp12)	Silent mutation
SNP	t15756a	N/A	1	100.0000%	ORF1ab (nsp12)	Silent mutation
SNP	g17144a	N/A	1	100.0000%	ORF1ab (nsp13)	R303H
SNP	c17410t	N/A	1	100.0000%	ORF1ab (nsp13)	R392C
SNP	a18163g	N/A	1	100.0000%	ORF1ab (nsp14)	I42V
SNP	a18492g	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
INS	21608[tcatgccgctgt]21609	N/A	+12	100.0000%	Spike	16[MPLF]17
SNP	c21618t	N/A	1	100.0000%	Spike	T19I
SNP	c21622t	N/A	1	100.0000%	Spike	Silent mutation
SNP	g21624c	N/A	1	100.0000%	Spike	R21T
DEL	Δ21633-21641	N/A	-9	100.0000%	Spike	A27S ΔLPP (amino acids 24-26)
SNP	c21711t	N/A	1	100.0000%	Spike	S50L
DEL	Δ21765-21770	N/A	-6	100.0000%	Spike	ΔHV (amino acids 69-70)
SNP	g21941t	N/A	1	100.0000%	Spike	V127F
SNP	g21987a	N/A	1	100.0000%	Spike	G142D
DEL	Δ21991-21993	N/A	-3	100.0000%	Spike	ΔY144
SNP	t22032c	N/A	1	100.0000%	Spike	F157S
SNP	c22033a	N/A	1	100.0000%		
SNP	a22034g	N/A	1	100.0000%	Spike	R158G
SNP	g23222a	N/A	1	100.0000%	Spike	E554K
SNP	c23271t	N/A	1	100.0000%	Spike	A570V
SNP	a23403g	N/A	1	100.0000%	Spike	D614G
SNP	c23423t	N/A	1	100.0000%	Spike	P621S
SNP	c23525t	N/A	1	100.0000%	Spike	H655Y

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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	t23599g	N/A	1	100.0000%	Spike	N679K
SNP	c23604a	N/A	1	100.0000%	Spike	P681R
SNP	c23854a	N/A	1	100.0000%	Spike	N764K
SNP	g23948t	N/A	1	100.0000%	Spike	D796Y
SNP	c24378t	N/A	1	100.0000%	Spike	S939F
SNP	a24424t	N/A	1	100.0000%	Spike	Q954H
SNP	t24469a	N/A	1	100.0000%	Spike	N969K
SNP	c24990t	N/A	1	100.0000%	Spike	P1143L
SNP	c25000t	N/A	1	100.0000%	Spike	Silent mutation
SNP	c25207t	N/A	1	100.0000%	Spike	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	g26529c	N/A	1	100.0000%	Membrane	D3H
SNP	c26577g	N/A	1	100.0000%	Membrane	Q19E
SNP	a26610g	N/A	1	100.0000%	Membrane	T30A
SNP	c26681t	N/A	1	100.0000%	Membrane	Silent mutation
SNP	g26709a	N/A	1	100.0000%	Membrane	A63T
SNP	c26833t	N/A	1	100.0000%	Membrane	A104V
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	a28271t	N/A	1	100.0000%	Intergenic (ORF8/ Nucleocapsid)	Untranslated
SNP	c28311t	N/A	1	100.0000%	Nucleocapsid	P13L
DEL	Δ28362-28370	N/A	-9	100.0000%	Nucleocapsid	ΔERS (amino acids 31-33)
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	c28958a	N/A	1	100.0000%	Nucleocapsid	Q229K
SNP	c29284t	N/A	1	100.0000%	Nucleocapsid	Silent mutation
SNP	a29510c	N/A	1	100.0000%	Nucleocapsid	S413R
DEL	29902	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 coverage. All variants in Table II are mismatches 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 the majority of SNPs.

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