

SARS-Related Coronavirus 2, Isolate hCoV-19/USA/MD-HP49081/2023 (Lineage DV.7.1) in VTA Cells

Catalog No. NR-59702

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/USA/MD-HP49081/2023 was isolated from a human in Maryland, USA, in 2023. NR-59702 lot 70067582 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% CO₂. The cells and supernatant were spin-clarified at 1500 × g for 10 minutes at 4°C.

Passage History:

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

Lot: 70067582

Manufacturing Date: 01APR2024

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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-HP49081/2023 (GISAID: EPI_ISL_18432211)	99.99% identity with isolate hCoV-19/USA/MD-HP49081/2023 (GISAID: EPI_ISL_18432211)
Titer by TCID₅₀ Assay in VTA Cells by Cytopathic Effect^{1,2} (8 days at 37°C and 5% CO ₂)	Report results	4.4 × 10 ⁶ TCID ₅₀ /mL
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 Blood agar, 37°C, aerobic Blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C and 5% CO ₂	No growth No growth No growth No growth No growth No growth No growth	Pending Pending Pending Pending Pending Pending Pending
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 (8.9 × 10⁶ per mL, 2.8 × 10⁶ per mL and 1.6 × 10⁶ per mL). 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.

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05 JUN 2024

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APPENDIX I: NGS Information for NR-59702 lot 70067582

Sequence analysis using AMGP readsQC-illumina.py pipeline and variant caller LoFreq version: 2.1.5 resulted in the discovery of two SNPs when compared to modified reference sequence GISAID EPI_ISL_18432211 (see Table I below). Additionally, both the reference sequence GISAID EPI_ISL_18432211 and NR-59702 lot 70067582 contained one hundred and ten SNPs and six DELs 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-59702 lot 70067582 and modified reference sequence GISAID EPI_ISL_18432211

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	c11750t	1429	1	99.6501%	ORF1ab (nsp6)	L260F
SNP	a19886g	1287	1	99.9223%	ORF1ab (nsp15)	K89R

Table II: Variants with different nucleotides between NR-59702 lot 70067582 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	t670g	N/A	1	100.0000%	ORF1ab (nsp1)	S135R
DEL	Δ686-694	N/A	-9	100.0000%	ORF1ab (nsp1)	ΔKSF (amino acids 141-143)
SNP	c1545t	N/A	1	100.0000%	ORF1ab (nsp2)	A247V
SNP	c2147t	N/A	1	100.0000%	ORF1ab (nsp2)	L448F
SNP	c2227t	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	c3796t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c3857a	N/A	1	100.0000%	ORF1ab (nsp3)	Q380K
SNP	c3927t	N/A	1	100.0000%	ORF1ab (nsp3)	S403L
SNP	t4036a	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	t4402a	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation

Variant Type	Variant Position and Identified Alternative Base	Coverage ¹	Length of Variant	Frequency of Variant ¹	Gene (Region)	Amino Acid Mutation
SNP	c4586t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c5183t	N/A	1	100.0000%	ORF1ab (nsp3)	P822S
SNP	c7162t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c7860t	N/A	1	100.0000%	ORF1ab (nsp3)	T1714I
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	c9866t	N/A	1	100.0000%	ORF1ab (nsp4)	L438F
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	c10908t	N/A	1	100.0000%	ORF1ab (nsp5)	A285V
DEL	Δ11288-11296	N/A	-9	100.0000%	ORF1ab (nsp6)	ΔSGF (amino acids 106-108)
SNP	a12444g	N/A	1	100.0000%	ORF1ab (nsp8)	N118S
SNP	c12750t	N/A	1	100.0000%	ORF1ab (nsp9)	A22V
SNP	c12880t	N/A	1	100.0000%	ORF1ab (nsp9)	Silent mutation
SNP	c14408t	N/A	1	100.0000%	ORF1ab (nsp12)	P323L
SNP	c15026t	N/A	1	100.0000%	ORF1ab (nsp12)	A529V
SNP	g15451a	N/A	1	100.0000%	ORF1ab (nsp12)	G671S
SNP	c15714t	N/A	1	100.0000%	ORF1ab (nsp12)	Silent mutation
SNP	c17410t	N/A	1	100.0000%	ORF1ab (nsp13)	R392C
SNP	a18163g	N/A	1	100.0000%	ORF1ab (nsp14)	I42V
SNP	g18583a	N/A	1	100.0000%	ORF1ab (nsp14)	V182I
SNP	c19955t	N/A	1	100.0000%	ORF1ab (nsp15)	T112I
SNP	a20055g	N/A	1	100.0000%	ORF1ab (nsp15)	Silent mutation
SNP	a20741g	N/A	1	100.0000%	ORF1ab (nsp16)	Q28R
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	g21987a	N/A	1	100.0000%	Spike	G142D
SNP	a22001g	N/A	1	100.0000%	Spike	K147E
SNP	t22016c	N/A	1	100.0000%	Spike	W152R
SNP	c22033a	N/A	1	100.0000%	Spike	F157L
SNP	a22115g	N/A	1	100.0000%	Spike	N185D
SNP	a22190g	N/A	1	100.0000%	Spike	I210V
SNP	t22200g	N/A	1	100.0000%	Spike	V213G
SNP	g22331a	N/A	1	100.0000%	Spike	G257S

Variant Type	Variant Position and Identified Alternative Base	Coverage ¹	Length of Variant	Frequency of Variant ¹	Gene (Region)	Amino Acid Mutation
SNP	g22577c	N/A	1	100.0000%	Spike	G339H
SNP	g22578a	N/A	1	100.0000%		
SNP	g22599c	N/A	1	100.0000%	Spike	R346T
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	a22893c	N/A	1	100.0000%	Spike	K444T
SNP	g22898a	N/A	1	100.0000%	Spike	G446S
SNP	t22917g	N/A	1	100.0000%	Spike	L452R
SNP	g22927t	N/A	1	100.0000%	Spike	L455F
SNP	t22928c	N/A	1	100.0000%	Spike	F456L
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	t23019c	N/A	1	100.0000%	Spike	F486S
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	c24134a	N/A	1	100.0000%	Spike	L858I
SNP	a24373g	N/A	1	100.0000%	Spike	Silent mutation
SNP	a24424t	N/A	1	100.0000%	Spike	Q954H
SNP	t24469a	N/A	1	100.0000%	Spike	N969K
SNP	t24991a	N/A	1	100.0000%	Spike	Silent mutation
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	g25606a	N/A	1	100.0000%	ORF3a	A72T

Variant Type	Variant Position and Identified Alternative Base	Coverage ¹	Length of Variant	Frequency of Variant ¹	Gene (Region)	Amino Acid Mutation
SNP	c25721t	N/A	1	100.0000%	ORF3a	A110V
SNP	t25959c	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
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	g27430a	N/A	1	100.0000%	ORF7a	A13T
SNP	c27807t	N/A	1	100.0000%	ORF7b	Silent mutation
SNP	t28098c	N/A	1	100.0000%	ORF8	S69P
SNP	a28271t	N/A	1	100.0000%	Intergenic (ORF8/ Nucleocapsid)	Untranslated
SNP	g28302a	N/A	1	100.0000%	Nucleocapsid	R10Q
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	a29510c	N/A	1	100.0000%	Nucleocapsid	S413R
SNP	a29558g	N/A	1	100.0000%	ORF10	M1V
DEL	Δ29734-29759	N/A	-26	100.0000%	3'UTR	Untranslated
DEL	29902	N/A	-1	100.0000%	3'UTR	Untranslated

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 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 between the modified 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.