

**SARS-Related Coronavirus 2, Isolate hCoV-19/USA/CA-Stanford-165\_S29/2023 (Lineage HK.3)**

**Catalog No. NR-59709**

**Product Description:**

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/USA/CA-Stanford-165\_S29/2023 was isolated from a human nasopharyngeal swab in California, USA, on September 9, 2023. NR-59709 lot 70066568 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<sub>2</sub>. The cells and supernatant were spin-clarified at 1500 × g for 10 minutes at 4°C.

**Passage History:**

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

**Lot: 70066568**

**Manufacturing Date: 04MAR2024**

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TEST	SPECIFICATIONS	RESULTS
<b>Identification by Infectivity in VTA Cells</b>	Cell rounding and detachment	Cell rounding and detachment
<b>Next-Generation Sequencing (NGS) of Complete Genome Using Illumina® MiSeq™ Platform</b> (Refer to Appendix I for NGS information)	≥ 98% identity with isolate hCoV-19/USA/CA-Stanford-165_S29/2023 (EPI_ISL_18403093)	99.98% identity with isolate hCoV-19/USA/CA-Stanford-165_S29/2023 (EPI_ISL_18403093)
<b>Titer by TCID<sub>50</sub> Assay in VTA Cells by Cytopathic Effect<sup>1,2</sup></b> (8 days at 37°C and 5% CO <sub>2</sub> )	Report results	1.4 × 10 <sup>6</sup> TCID <sub>50</sub> per mL
<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 Blood agar, 37°C, aerobic Blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C and 5% CO <sub>2</sub>	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 (1.6 × 10<sup>6</sup> per mL, 1.6 × 10<sup>6</sup> per mL and 8.9 × 10<sup>5</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.

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16 MAY 2024

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**APPENDIX I: NGS Information for NR-59709 lot 70066568**

Sequence analysis using AMGP readsQC-illumina.py pipeline and variant caller LoFreq version: 2.1.5 resulted in the discovery of four SNPs when compared to reference sequence GISAID EPI\_ISL\_18403093 (see Table I below). Additionally, both the reference sequence GISAID EPI\_ISL\_18403093 and NR-59711 lot 70066570 contained one hundred ten SNPs, and five 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-59709 lot 70066568 and reference sequence GISAID EPI\_ISL\_18403093**

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	c26542t	432	1	37.5000%	Membrane	T7I
SNP	g27758a	1391	1	10.5679%	ORF7b	M1I
SNP	t27760a	1410	1	10.4965%	ORF7b	I2N
SNP	t27761c	1445	1	11.0727%		

**Table II: Variants with different nucleotides between NR-59709 lot 70066568 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	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	c2334t	N/A	1	100.0000%	ORF1ab (nsp2)	A510V
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	c5575t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	g5720a	N/A	1	100.0000%	ORF1ab (nsp3)	G1001S
SNP	c6541t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c6695t	N/A	1	100.0000%	ORF1ab (nsp3)	P1326S
SNP	c9344t	N/A	1	100.0000%	ORF1ab (nsp4)	L264F
SNP	a9424g	N/A	1	100.0000%	ORF1ab (nsp4)	Silent mutation

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	c9448a	N/A	1	100.0000%	ORF1ab (nsp4)	Silent mutation
SNP	c9534t	N/A	1	100.0000%	ORF1ab (nsp4)	T327I
SNP	c9693t	N/A	1	100.0000%	ORF1ab (nsp4)	A380V
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
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	c12880t	N/A	1	100.0000%	ORF1ab (nsp9)	Silent mutation
SNP	g13627a	N/A	1	100.0000%	ORF1ab (nsp12)	D63N
SNP	c14408t	N/A	1	100.0000%	ORF1ab (nsp12)	P323L
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	a16878t	N/A	1	100.0000%	ORF1ab (nsp13)	Silent mutation
SNP	c17410t	N/A	1	100.0000%	ORF1ab (nsp13)	R392C
SNP	c17502t	N/A	1	100.0000%	ORF1ab (nsp13)	Silent mutation
SNP	t17859c	N/A	1	100.0000%	ORF1ab (nsp13)	Silent mutation
SNP	a18163g	N/A	1	100.0000%	ORF1ab (nsp14)	I42V
SNP	a18492g	N/A	1	100.0000%	ORF1ab (nsp14)	Silent mutation
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	g21718t	N/A	1	100.0000%	Spike	Q52H
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	c22480t	N/A	1	100.0000%	Spike	Silent mutation

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	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	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	g22927t	N/A	1	100.0000%	Spike	L455F
SNP	t22930a	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	g23006c	N/A	1	100.0000%	Spike	G482R
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

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	c25572t	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
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	a27507c	N/A	1	100.0000%	ORF7a	Silent mutation
SNP	c27807t	N/A	1	100.0000%	ORF7b	Silent mutation
SNP	g27915t	N/A	1	100.0000%	ORF8	G8stop <sup>2</sup>
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
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	c29625t	N/A	1	100.0000%	ORF10	S23F
DEL	29902	N/A	-1	100.0000%	3'UTR	Untranslated

<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 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.

<sup>2</sup>ORF8 G8stop results in 94.2% truncation of the protein sequence.