Certificate of Analysis for NR-58716

SARS-Related Coronavirus 2, Isolate hCoV-19/USA/MD-HP34985/2022 (Lineage BF.5; Omicron Variant)

Catalog No. NR-58716

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/USA/MD-HP34985/2022 was isolated from a human on July 11, 2022, in Maryland, USA. NR-58716 lot 70055929 was produced by infecting *Cercopithecus aethiops* Kidney Epithelial Cells Expressing Transmembrane Protease, Serine 2 and Human Angiotensin-Converting Enzyme 2 (Vero E6-TMPRSS2-T2A-ACE2; VTA; BEI Resources lot 70050994) 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% 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 = *Cercopithecus aethiops* kidney cells with transmembrane protease, serine 2 gene (Vero E6-TMPRSS2); VTA = *Cercopithecus aethiops* kidney cells with transmembrane protease, serine 2 gene and human angiotensin-converting enzyme 2 (Vero E6-TMPRSS2-T2A-ACE2)

Lot: 70055929 **Manufacturing Date: 26SEP2022**

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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® iSeq™ 100 Platform (Refer to Appendix I for NGS information)	≥ 98% identity with isolate hCoV-19/USA/MD-HP34985/2022 (GISAID: EPI_ISL_14077403)	99.98% identity with isolate hCoV-19/USA/MD-HP34985/2022 (GISAID: EPI_ISL_14077403)
Titer by $TCID_{50}$ Assay in VTA Cells by Cytopathic Effect[1] (8 days at 37°C and 5% CO_2)	Report results	2.4×10^7 $TCID_{50}$ per mL[2]
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic[3]	No growth	No growth
Trypticase Soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, aerobic	No growth	No growth
Sheep blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
DMEM with 10% FBS, 37°C, aerobic	No growth	No growth
Mycoplasma Contamination		
Agar and broth culture (14-day incubation at 37°C)	None detected	None detected
DNA detection by PCR of extracted Test Article nucleic acid	None detected	None detected

[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 (2.8×10^7 per mL, 2.8×10^7 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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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.

APPENDIX I: NGS Information for NR-58716 lot 70055929

Sequence analysis using AMGP readsQC-illumina.py pipeline and variant caller LoFreq version: 2.1.5 resulted in the discovery of three SNPs when compared to GISAID EPI_ISL_14077403 (see Table I below). Additionally, both the reference sequence EPI_ISL_14077403 and NR-58716 lot 70055929 contained seventy-three 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-58716 lot 70055929 and reference sequence EPI_ISL_14077403

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	a11448g	2640	1	33.1818%	ORF1ab (nsp6)	D159G
SNP	t11463a	2275	1	12.8352%	ORF1ab (nsp6)	M164K
SNP	c13164t	1810	1	44.5304%	ORF1ab (nsp10)	T47I

Table II: Variants with different nucleotides between NR-58716 lot 70055929 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
SNP	c1627t	N/A	1	100.0000%	ORF1ab (nsp2)	Silent mutation
SNP	g2177t	N/A	-9	100.0000%	ORF1ab (nsp2)	G458C
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	t6979g	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
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	c9967t	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

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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
DEL	Δ11288-11296	N/A	-9	100.0000%	ORF1ab (nsp6)	ΔSGF (amino acids 106-108)
SNP	g12160a	N/A	1	100.0000%	ORF1ab (nsp8)	Silent mutation
SNP	c12880t	N/A	1	100.0000%	ORF1ab (nsp9)	Silent mutation
SNP	c13821t	N/A	1	100.0000%	ORF1ab (nsp12)	Silent mutation
SNP	c14408t	N/A	1	100.0000%	ORF1ab (nsp12)	P323L
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	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)
DEL	Δ21765-21770	N/A	-6	100.0000%	Spike	ΔHV (amino acids 69-70)
SNP	g21987a	N/A	1	100.0000%	Spike	G142D
SNP	t22200g	N/A	1	100.0000%	Spike	V213G
SNP	g22578a	N/A	1	100.0000%	Spike	G339D
SNP	c22674t	N/A	1	100.0000%	Spike	S371P
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	t22917g	N/A	1	100.0000%	Spike	L452R
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	t23018g	N/A	1	100.0000%	Spike	F486V
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	H665Y
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

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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	a24424t	N/A	1	100.0000%	Spike	Q954H
SNP	t24469a	N/A	1	100.0000%	Spike	N969K
SNP	g24620t	N/A	1	100.0000%	Spike	A1020S
SNP	c25000t	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	T91I
SNP	g26529a	N/A	1	100.0000%	Membrane	D3N
SNP	c26577g	N/A	1	100.0000%	Membrane	Q19E
SNP	g26709a	N/A	1	100.0000%	Membrane	A63T
SNP	a27038g	N/A	1	100.0000%	Membrane	Silent mutation
SNP	c27532t	N/A	1	100.0000%	ORF7a	H47Y
SNP	c27807t	N/A	1	100.0000%	ORF7b	Silent mutation
SNP	c27889t	N/A	1	100.0000%	Intergenic (ORF7b-ORF8)	Untranslated
SNP	a28271t	N/A	1	100.0000%	Intergenic (ORF8-Nucleocapsid)	Untranslated
SNP	c28311t	N/A	1	100.0000%	Nucleocapsid	P13L
SNP	a28330g	N/A	1	100.0000%	Nucleocapsid	Silent mutation
DEL	Δ28362-28370	N/A	-9	100.0000%	Nucleocapsid	ΔERS (amino acids 31-33)
SNP	a28402g	N/A	1	100.0000%	Nucleocapsid	Silent mutation
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

[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.

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