Certificate of Analysis for NR-55698

SARS-Related Coronavirus 2, Isolate hCoV-19/USA/WI-UW-4340/2021 (Lineage B.1.621)

Catalog No. NR-55698

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/USA/WI-UW-4340/2021 was isolated from a human nasopharyngeal swab on April 22, 2021, in Madison, Wisconsin, USA. NR-55698 lot 70046989 was produced by infecting *Homo sapiens* lung adenocarcinoma epithelial cells (Calu-3; ATCC® HTB-55™) 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)/C(1) (University of Wisconsin-Madison/BEI Resources); VT = *Cercopithecus aethiops* kidney cells expressing TMPRSS2; C = Calu-3

Lot: 70046989 Manufacturing Date: 20AUG2021

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TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Calu-3 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 hCoV-19/USA/WI-UW-4340/2021 (GISAID: EPI_ISL_1827552)	99.98% identity with hCoV-19/USA/WI-UW-4340/2021 (GISAID: EPI_ISL_1827552)
Titer by $TCID_{50}$ Assay in Calu-3 Cells by Cytopathic Effect[1] (7 days at 37°C and 5% CO_2)	Report results	4.4×10^6 $TCID_{50}$ 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 (2.8×10^6 per mL, 1.6×10^6 per mL and 8.9×10^6 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.

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

/Sonia Bjorum Brower/
Sonia Bjorum Brower 08 AUG 2022
Lead Technical Writer or designee, ATCC Federal Solutions

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Support Provided by NIAID

APPENDIX I: NGS Information for NR-55698 lot 70046989

Sequence analysis using AMGP readsQC-illumina.py pipeline and variant callers LoFreq version: 2.1.5 and freebayes version: v1.3.1-dirty resulted in the discovery of three SNPs when compared to the reference sequence from GISAID EPI_ISL_1827552 (see Table I below). Additionally, both the reference sequence EPI_ISL_1827552 and NR-55698 lot 70046989 contained thirty-one SNPs and one deletion (DEL) when compared to GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) (see Table II below). At nucleotide 21995, NR-556978 lot 7004989 has an insertion (INS) whereas the reference sequence EPI_ISL_1827552 has a DEL (see Table III below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Table I: Variants with different nucleotides between NR-55698 lot 70046989 and reference sequence EPI_ISL_1827552

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	t13542g	708	1	6.4972%	ORF1ab (nsp12)	Silent mutation
SNP	t14679c	977	1	5.9365 %	ORF1ab (nsp12)	Silent mutation
SNP	t17895c	1226	1	6.7700%	ORF1ab (nsp13)	Silent mutation

Table II: Variants with different nucleotides between NR-55698 lot 70046989 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	c3037t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	a3428g	N/A	1	100.0000%	ORF1ab (nsp3)	T237A
SNP	c4878t	N/A	1	100.0000%	ORF1ab (nsp3)	T720I
SNP	c6037t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c10029t	N/A	1	100.0000%	ORF1ab (nsp4)	T492I
SNP	a11451g	N/A	1	100.0000%	ORF1ab (nsp6)	Q160R
SNP	a13057t	N/A	1	100.0000%	ORF1ab (nsp10)	Silent mutation
SNP	c14408t	N/A	1	100.0000%	ORF1ab (nsp12)	P323L
SNP	c17491t	N/A	1	100.0000%	ORF1ab (nsp13)	P419S
SNP	c18877t	N/A	1	100.0000%	ORF1ab (nsp14)	Silent mutation
SNP	t19035c	N/A	1	100.0000%	ORF1ab (nsp14)	Silent mutation
SNP	c20148t	N/A	1	100.0000%	ORF1ab (nsp15)	Silent mutation
SNP	c21846t	N/A	1	100.0000%	Spike	T95I
SNP	t21992a	N/A	1	100.0000%	Spike	Y144T

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www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

Variant Type	Variant Position and Identified Alternative Base	Coverage[1]	Length of Variant	Frequency of Variant[1]	Gene (Region)	Amino Acid Mutation
SNP	a21993c	N/A	1	100.0000%		
SNP	g22599a	N/A	1	100.0000%	Spike	R346K
SNP	g23012a	N/A	1	100.0000%	Spike	E484K
SNP	a23063t	N/A	1	100.0000%	Spike	N501Y
SNP	a23403g	N/A	1	100.0000%	Spike	D614G
SNP	c23604a	N/A	1	100.0000%	Spike	P681H
SNP	g24410a	N/A	1	100.0000%	Spike	D950N
SNP	c24784a	N/A	1	100.0000%	Spike	N1074K
SNP	g25563t	N/A	1	100.0000%	ORF3a	Q57H
DEL	Δ26158-26161	N/A	-4	100.0000%	ORF3a	V256I, N257Q, Δ amino acids 258-275
SNP	a26492t	N/A	1	100.0000%	Intergenic-Envelope/Membrane	Untranslated
SNP	g27758t	N/A	1	100.0000%	ORF7b	M1I
SNP	c27925a	N/A	1	100.0000%	ORF8	T11K
SNP	c28005t	N/A	1	100.0000%	ORF8	P38S
SNP	c28093t	N/A	1	100.0000%	ORF8	S67F
SNP	a28272t	N/A	1	100.0000%	Intergenic (ORF8/Nucleocapsid)	Untranslated
SNP	c28887t	N/A	1	100.0000%	Nucleocapsid	T205I

[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.

Table III: Variants with different nucleotides between NR-55698 lot 70046989, reference sequence EPI_ISL_1827552 and GenBank MN908947.3 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome)

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
INS	21995[cta]21996[1]	N/A	+3	100.0000%	Spike	Y145S[N]H146

[1]At this location, the reference EPI_ISL_1827552 has a four-nucleotide insertion (21995[ntta]21996) compared to GenBank MN908947.3 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome), while NR-55698 lot 70046989 consensus sequence has a three-nucleotide insertion (21995[cta]21996).

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Fax: 703-365-2898