Certificate of Analysis for NR-55697

SARS-Related Coronavirus 2, Isolate hCoV-19/USA/WI-IRI-001/2021 (Lineage B.1.630)

Catalog No. NR-55697

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/USA/WI-IRI-001/2021 was isolated from a nasal swab from a human in Madison, Wisconsin, USA, in July 2021. NR-55697 lot 70047985 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 4 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 with transmembrane protease, serine 2 gene (TMPRSS2); C = Calu-3

Lot: 70047985 Manufacturing Date: 12OCT2021

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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 SARS-CoV-2, hCoV-19 lineage B.1.630 (GISAID: EPI_ISL_3190270)	99.99% identity with SARS-CoV-2, hCoV-19 lineage B.1.630 (GISAID: EPI_ISL_3190270)
Titer by $TCID_{50}$ Assay in Calu-3 Cells by Cytopathic Effect[1] (7 days at 37°C and 5% CO_2)	Report results	1.6×10^5 $TCID_{50}$ 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 (1.6×10^5 per mL, 1.6×10^5 per mL and 1.6×10^5 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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APPENDIX I: NGS Information for NR-55697 lot 70047985

Note: The reference sequence used in this analysis, EPI_ISL_3190270, differs from EPI_ISL_5335935, the passage 1 sequence submitted for this virus. Due to quality issues in EPI_ISL_5335935, this sequence was not ideal for comparing sequence of NR-55697. Selection of EPI_ISL_3190270 as the reference sequence was accomplished by mapping the in-house sequence reads to the Wuhan-Hu-1 sequence, generating a consensus sequence, and submitting that sequence to the GISAID Audacity Instant tool to find the most closely related sequence. While EPI_ISL_3190270 and EPI_ISL_5335935 are different, both belong to the Pango Lineage B.1.630, and all of the amino acid mutations characteristic of this lineage are present in the NR-55697 sequence.

Sequence analysis of NR-55697 using SBC v2.0 pipeline and freebayes v1.3.1 variant caller resulted in the discovery of four SNPs when compared to the reference sequence EPI_ISL_3190270 (see Table I below). Additionally, both the reference sequence EPI_ISL_3190270 and NR-55697 lot 70047985 contained thirty-six SNPs and four deletions (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-55697 lot 70047985 and reference sequence EPI_ISL_3190270

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	a3625g	350	1	5.4286%	ORF1ab (nsp3)	Silent mutation
SNP	g10364a	634	1	80.7571%	ORF1ab (nsp5)	V104I
SNP	c11355t	384	1	8.0729%	ORF1ab (nsp6)	A128V
SNP	g11855t	378	1	7.6720%	ORF1ab (nsp7)	D5Y

Table II: Variants with different nucleotides between NR-55697 lot 70047985 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	c629t	N/A	1	100.0000%	ORF1ab (nsp1)	L122F
SNP	c1059t	N/A	1	100.0000%	ORF1ab (nsp2)	T85I
SNP	a1262g	N/A	1	100.0000%	ORF1ab (nsp2)	T153A
SNP	c1627t	N/A	1	100.0000%	ORF1ab (nsp2)	Silent mutation
SNP	c3037t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	g4963t	N/A	1	100.0000%	ORF1ab (nsp3)	R748S
SNP	a5040g	N/A	1	100.0000%	ORF1ab (nsp3)	Q774R
SNP	a5648c	N/A	1	100.0000%	ORF1ab (nsp3)	K977Q
SNP	c6730t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c6794t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c7768t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c9891t	N/A	1	100.0000%	ORF1ab (nsp4)	A446V
DEL	Δ11288-11296	N/A	-9	100.0000%	ORF1ab (nsp6)	ΔSGF (aa106-108)
SNP	g11855t	N/A	1	100.0000%	ORF1ab (nsp7)	D5Y

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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	c14408t	N/A	1	100.0000%	ORF1ab (nsp12)	P323L
SNP	t17505c	N/A	1	100.0000%	ORF1ab (nsp13)	Silent mutation
SNP	c19602t	N/A	1	100.0000%	ORF1ab (nsp14)	Silent mutation
SNP	g21255t	N/A	1	100.0000%	ORF1ab (nsp16)	Silent mutation
SNP	c21588t	N/A	1	100.0000%	Spike	P9L
SNP	c21637t	N/A	1	100.0000%	Spike	Silent mutation
SNP	g21969t	N/A	1	100.0000%	Spike	C136F
DEL	Δ21991-21993	N/A	-3	100.0000%	Spike	ΔY (amino acid 144)
SNP	c22227t	N/A	1	100.0000%	Spike	A222V
DEL	Δ22289-22294	N/A	-6	100.0000%	Spike	ΔAL (amino acids 243-244)
SNP	t22917g	N/A	1	100.0000%	Spike	L452R
SNP	c22995g	N/A	1	100.0000%	Spike	T478R
SNP	g23012c	N/A	1	100.0000%	Spike	E484Q
SNP	a23403g	N/A	1	100.0000%	Spike	D614G
SNP	c23525t	N/A	1	100.0000%	Spike	H655Y
SNP	g24410a	N/A	1	100.0000%	Spike	D950N
DEL	Δ25498-25530	N/A	-33	100.0000%	ORF3a	ΔPIQASLPFGWL (amino acids 36-46)
SNP	g25563t	N/A	1	100.0000%	ORF3a	Q57H
SNP	t27796c	N/A	1	100.0000%	ORF7b	L14S
SNP	c27889t	N/A	1	100.0000%	Intergenic (ORF7b/ORF8)	Untranslated
SNP	a28272g	N/A	1	100.0000%	Intergenic (ORF8/ nucleocapsid)	Untranslated
SNP	c28512g	N/A	1	100.0000%	Nucleocapsid	P80R
SNP	c28887t	N/A	1	100.0000%	Nucleocapsid	T205I
SNP	g29508a	N/A	1	100.0000%	Nucleocapsid	S412N
SNP	c29739t	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 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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