Certificate of Analysis for NR-56309

SARS-Related Coronavirus 2, Isolate hCoV-19/USA/VA-NIDDL-48553/2021 (Lineage B.1.637)

Catalog No. NR-56309

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/USA/VA-NIDDL-48553/2021 was isolated from a human nasopharyngeal swab on April 23, 2021, in Virginia, USA. NR-56309 lot 70050195 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) (Naval Medical Research Center/BEI Resources); VT = *Cercopithecus aethiops* kidney cells expressing transmembrane protease, serine 2; C = Calu-3

Lot: 70050195 Manufacturing Date: 31JAN2022

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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 isolate hCoV-19/USA/VA-NIDDL-48553/2021 (GISAID: EPI_ISL_2533889)	99.99% identity with isolate hCoV-19/USA/VA-NIDDL-48553/2021 (GISAID: EPI_ISL_2533889)
Titer by $TCID_{50}$ Assay in Calu-3 Cells by Cytopathic Effect[1] (6 days at 37°C and 5% CO_2)	Report results	9.2×10^6 $TCID_{50}$ 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, 8.9×10^6 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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APPENDIX I: NGS Information for NR-56309 lot 70050195

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 two SNPs when compared to the reference sequence from GISAID EPI_ISL_2533889 (see Table I below). Additionally, both the reference sequence EPI_ISL_2533889 and NR-56309 lot 70050195 contained twenty-nine SNPs and three 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-56309 lot 70050195 and reference sequence EPI_ISL_2533889

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	c2536t	1628	1	19.1646%	ORF1ab (nsp2)	Silent mutation
SNP	t22114c	803	1	5.6040 %	Spike	Silent mutation

Table II: Variants with different nucleotides between NR-56309 lot 70050195 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	c1059t	N/A	1	100.0000%	ORF1ab (nsp2)	T85I
SNP	c2189t	N/A	1	100.0000%	ORF1ab (nsp2)	L462F
SNP	c3037t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	g3685a	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c5581t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c6468t	N/A	1	100.0000%	ORF1ab (nsp3)	T1250I
SNP	a9749g	N/A	1	100.0000%	ORF1ab (nsp4)	K399E
SNP	t9867c	N/A	1	100.0000%	ORF1ab (nsp4)	L438P
SNP	c9891t	N/A	1	100.0000%	ORF1ab (nsp4)	A446V
DEL	Δ11288-11296	N/A	-9	100.0000%	ORF1ab (nsp6)	ΔSGF (amino acids106-108)
SNP	g11804a	N/A	1	100.0000%	ORF1ab (nsp6)	V278I
SNP	a12030g	N/A	1	100.0000%	ORF1ab (nsp7)	Q63R
SNP	c14408t	N/A	1	100.0000%	ORF1ab (nsp12)	P323L
SNP	t19452c	N/A	1	100.0000%	ORF1ab (nsp14)	Silent mutation
SNP	a21801g	N/A	1	100.0000%	Spike	D80G

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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	Δ21991-21993	N/A	-3	100.0000%	Spike	ΔY144
SNP	t22032c	N/A	1	100.0000%	Spike	F157S
SNP	t22917g	N/A	1	100.0000%	Spike	L452R
SNP	a23403g	N/A	1	100.0000%	Spike	D614G
SNP	c24138a	N/A	1	100.0000%	Spike	T859N
SNP	g24410c	N/A	1	100.0000%	Spike	D950H
SNP	c25413t	N/A	1	100.0000%	ORF3a	Silent mutation
SNP	c25517t	N/A	1	100.0000%	ORF3a	P42L
SNP	g25563t	N/A	1	100.0000%	ORF3a	Q57H
SNP	c25703t	N/A	1	100.0000%	ORF3a	P104L
SNP	g27260a	N/A	1	100.0000%	ORF6	R20K
SNP	c27925t	N/A	1	100.0000%	ORF8	T11I
SNP	g28044t	N/A	1	100.0000%	ORF8	A51S
DEL	Δ28271	N/A	-1	100.0000%	Intergenic – ORF8/Nucleocapsid	Untranslated
SNP	c28887t	N/A	1	100.0000%	Nucleocapsid	T205I
SNP	g28975a	N/A	1	100.0000%	Nucleocapsid	M234I

[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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