Certificate of Analysis for NR-56695

SARS-Related Coronavirus 2, Isolate hCoV-19/France/PAC-IHUMI-6070/2022 (Lineage AY.4 plus omicron; XD Variant)

Catalog No. NR-56695

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/France/PAC-IHUMI-6070/2022 was isolated from a human on February 9, 2022, in France. NR-56695 lot 70051973 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:

V(1)/C(1) (Hospital-University Institute/BEI Resources); V = *Cercopithecus aethiops* kidney cells; C = Calu-3

Lot: 70051973 Manufacturing Date: 25APR2022

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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/France/PAC-IHUMI-6070/2022 (GISAID: EPI_ISL_10640045)	99.94% identity with SARS-CoV-2, hCoV-19/France/PAC-IHUMI-6070/2022 (GISAID: EPI_ISL_10640045)
Titer by $TCID_{50}$ Assay in Calu-3 Cells by Cytopathic Effect[1] (8 days at 37°C and 5% CO_2)	Report results	1.6×10^6 $TCID_{50}$ per mL
Endotoxin Content (*Limulus* Amoebocyte Lysate Assay)	Report results	≤ 0.03 EU per mL
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic[2]	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]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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Sonia Bjorum Brower 28 SEP 2022
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-56695 lot 70051973

Note: The sequence submitted to GISAID for this isolate (EPI_ISL_10640045) contains stretches of ambiguous nucleotides (Ns), which required selection of an alternate sequence to serve as a suitable reference for comparison of the sequence from NR-56695. The reference sequence used for quality control testing and reporting purposes is a modified sequence of EPI_ISL_10528736 in which the ambiguous Ns are replaced by the corresponding nucleotides from the Wuhan-Hu-1 sequence. None of the reported amino acid mutations occurred in the corrected regions.

Sequence analysis using AMGP readsQC-illumina.py and variant caller LoFreq version: 2.1.5 resulted in the discovery of one insertion (INS) when compared to EPI_ISL_10528736 (see Table I below). Additionally, both the reference sequence EPI_ISL_10528736 and NR-56695 lot 70051973 contained sixty-seven 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-56695 lot 70051973 and reference sequence EPI_ISL_10528736

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
INS	Δ22204 [tgagccaga] 22205	448	+9	28.3482%	Spike	D215STOP, Δamino acids 216- 1274

Table II: Variants with different nucleotides between NR-56695 lot 70051973 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	g210t	N/A	1	100.0000%	5'UTR	Untranslated
SNP	c241t	N/A	1	100.0000%	5'UTR	Untranslated
SNP	a1321c	N/A	1	100.0000%	ORF1ab (nsp2)	E172D
SNP	c2644t	N/A	1	100.0000%	ORF1ab (nsp2)	Silent mutation
SNP	c3037t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	g4181t	N/A	1	100.0000%	ORF1ab (nsp3)	A488S
SNP	c6402t	N/A	1	100.0000%	ORF1ab (nsp3)	P1228L
SNP	c7124t	N/A	1	100.0000%	ORF1ab (nsp3)	P1469S
SNP	c7851t	N/A	1	100.0000%	ORF1ab (nsp3)	A1711V
SNP	a8723g	N/A	1	100.0000%	ORF1ab (nsp4)	I57V
SNP	c8986t	N/A	-9	100.0000%	ORF1ab (nsp4)	Silent mutation
SNP	g9053t	N/A	1	100.0000%	ORF1ab (nsp4)	V167L
SNP	c10029t	N/A	1	100.0000%	ORF1ab (nsp4)	T492I

Variant Type	Variant Position and Identified Alternative Base	Coverage[1]	Length of Variant	Frequency of Variant[1]	Gene (Region)	Amino Acid Mutation
SNP	a11201g	N/A	1	100.0000%	ORF1ab (nsp6)	T77A
SNP	a11332g	N/A	1	100.0000%	ORF1ab (nsp6)	Silent mutation
SNP	g12038a	N/A	1	100.0000%	ORF1ab (nsp7)	V66I
SNP	c14407t	N/A	1	100.0000%	ORF1ab (nsp12)	P323F
SNP	c14408t	N/A	1	100.0000%		
SNP	t15264c	N/A	1	100.0000%	ORF1ab (nsp12)	Silent mutation
SNP	g15451a	N/A	1	100.0000%	ORF1ab (nsp12)	G671S
SNP	c16466t	N/A	1	100.0000%	ORF1ab (nsp13)	P77L
SNP	c19220t	N/A	1	100.0000%	ORF1ab (nsp14)	A394V
SNP	c21618g	N/A	1	100.0000%	Spike	T19R
SNP	g21641t	N/A	1	100.0000%	Spike	A27S
SNP	c21846t	N/A	1	100.0000%	Spike	T95I
SNP	g21987a	N/A	1	100.0000%	Spike	G142D
DEL	Δ22029-22034	N/A	-6	100.0000%	Spike	ΔEF (amino acids 156-157), R158G
DEL	Δ22194-22196	N/A	-3	100.0000%	Spike	ΔN211, L212I
SNP	g22578a	N/A	1	100.0000%	Spike	G339D
SNP	t22673c	N/A	1	100.0000%	Spike	S371L
SNP	c22674t	N/A	1	100.0000%		
SNP	t22679c	N/A	1	100.0000%	Spike	S373P
SNP	c22686t	N/A	1	100.0000%	Spike	S375F
SNP	g22813t	N/A	1	100.0000%	Spike	K417N
SNP	t22882g	N/A	1	100.0000%	Spike	N440K
SNP	g22898a	N/A	1	100.0000%	Spike	G446S
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	a23040g	N/A	1	100.0000%	Spike	Q493R
SNP	g23048a	N/A	1	100.0000%	Spike	G496S
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	c23202a	N/A	1	100.0000%	Spike	T547K
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	c24130a	N/A	1	100.0000%	Spike	N856K

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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	c24503t	N/A	1	100.0000%	Spike	L981F
SNP	c25000t	N/A	1	100.0000%	Spike	Silent mutation
SNP	c25667t	N/A	1	100.0000%	ORF3a	S92L
SNP	g25855t	N/A	1	100.0000%	ORF3a	D155Y
SNP	t26767c	N/A	1	100.0000%	Membrane	I82T
SNP	t27638c	N/A	1	100.0000%	ORF7a	V82A
SNP	c27752t	N/A	1	100.0000%	ORF7a	T120I
SNP	c27874t	N/A	1	100.0000%	ORF7b	T40I
DEL	Δ28248-28253	N/A	-6	100.0000%	ORF8	ΔDF (amino acids 119-120)
DEL	28271	N/A	-1	100.0000%	Intergenic (ORF8/ Nucleocapsid)	Untranslated
SNP	a28461g	N/A	1	100.0000%	Nucleocapsid	D63G
SNP	g28881t	N/A	1	100.0000%	Nucleocapsid	R203M
SNP	g28916t	N/A	1	100.0000%	Nucleocapsid	G215C
SNP	g29402t	N/A	1	100.0000%	Nucleocapsid	D377Y
SNP	g29540a	N/A	1	100.0000%	Intergenic (Nucleocapsid /ORF10)	Untranslated
SNP	g29645t	N/A	1	100.0000%	ORF10	V30L
SNP	g29742t	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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