

SARS-Related Coronavirus 2, Isolate hCoV-19/Japan/TY41-716/2022 (Lineage BA.2.75; Omicron Variant)

Catalog No. NR-58706

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/Japan/TY41-716/2022 was isolated from a human with a history of travel to Asia/India on June 13, 2022, in Tokyo, Japan. NR-58706 lot 70055732 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) 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% CO₂. The cells and supernatant were spin-clarified at 1500 × g for 10 minutes at 4°C.

Passage History:

VT(1)/VTA(1) (National Institute of Infectious Diseases/BEI Resources); VT = *Cercopithecus aethiops* kidney epithelial cells expressing transmembrane protease, serine 2; VTA = *Cercopithecus aethiops* kidney epithelial cells expressing transmembrane protease, serine 2 and human angiotensin-converting enzyme 2

Lot: 70055732

Manufacturing Date: 09SEP2022

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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 hCoV-19/Japan/TY41-716/2022 (GISAID: EPI_ISL_13969765)	99.97% identity with hCoV-19/Japan/TY41-716/2022 (GISAID: EPI_ISL_13969765)
Titer by TCID₅₀ Assay in VTA Cells by Cytopathic Effect¹ (5 days at 37°C and 5% CO ₂)	Report results	9.2 × 10 ⁵ TCID ₅₀ per mL ²
Sterility (21-day incubation) Harpo's HTYE broth, 37°C and 26°C, aerobic ³ Trypticase Soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C, aerobic	No growth No growth No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth No growth No growth
Mycoplasma Contamination Agar and broth culture (14-day incubation at 37°C) DNA detection by PCR of extracted Test Article nucleic acid	None detected None detected	None detected None detected

¹The Tissue Culture Infectious Dose 50% (TCID₅₀) endpoint is the 50% infectious endpoint in cell culture. The TCID₅₀ 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% (LD₅₀) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID₅₀ provides a measure of the titer (or infectivity) of a virus preparation.

²Titer was determined by cytopathic effects (CPE) and completed in triplicate (2.8 × 10⁵ per mL, 8.9 × 10⁵ per mL and 1.6 × 10⁶ per mL). The average of the three values is reported.

³Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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15 JAN 2023

Technical Manager or designee, ATCC Federal Solutions

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APPENDIX I: NGS Information for NR-58706 lot 70055732

Sequence analysis using AMGP readsQC-illumina.py pipeline and variant caller LoFreq version: 2.1.5 resulted in the discovery of ten SNPs and one deletion (DEL) when compared to EPI_ISL_13969765 (see Table I below). Additionally, both EPI_ISL_13969765 and NR-58706 lot 70055732 contained eighty-two 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-58706 lot 70055732 and reference sequence EPI_ISL_13969765

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	t1963a	217	1	5.5300%	ORF1ab (nsp1)	Silent mutation
SNP	t1963g	217	1	6.9124%	ORF1ab (nsp1)	Silent mutation
SNP	a3625g	296	1	8.7838%	ORF1ab (nsp3)	Silent mutation
SNP	t11313g	279	1	5.3763%	ORF1ab (nsp6)	V114G
SNP	t14679a	155	1	6.4516%	ORF1ab (nsp12)	Silent mutation
SNP	t14679c	155	1	18.0645%	ORF1ab (nsp12)	Silent mutation
SNP	g17808a	635	1	9.4488%	ORF1ab (nsp13)	Silent mutation
SNP	c23606t	513	1	8.3821%	Spike	R682W
SNP	a25806g	220	1	5.0000%	ORF3a	Silent mutation
DEL	26284- 26286	439	-3	22.3235%	Envelope	ΔV14
SNP	a26369g	366	1	10.3825%	Envelope	Y42C

Table II: Variants with different nucleotides between NR-58706 lot 70055732 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	t670g	N/A	1	100.0000%	ORF1ab (nsp1)	S135R
SNP	c2790t	N/A	1	100.0000%	ORF1ab (nsp3)	T24I
SNP	c3037t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c3796t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c3927t	N/A	1	100.0000%	ORF1ab (nsp3)	S403L
SNP	g4184a	N/A	1	100.0000%	ORF1ab (nsp3)	G489S

Variant Type	Variant Position and Identified Alternative Base	Coverage ¹	Length of Variant	Frequency of Variant ¹	Gene (Region)	Amino Acid Mutation
SNP	c4321t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c4586t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c5183t	N/A	1	100.0000%	ORF1ab (nsp3)	P822S
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	c9866t	N/A	1	100.0000%	ORF1ab (nsp4)	L438F
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
SNP	g11055t	N/A	1	100.0000%	ORF1ab (nsp6)	S28I
DEL	Δ11288-11296	N/A	-9	100.0000%	ORF1ab (nsp6)	ΔSGF (amino acids 106-108)
SNP	a12444g	N/A	1	100.0000%	ORF1ab (nsp8)	N118S
SNP	c12880t	N/A	1	100.0000%	ORF1ab (nsp9)	Silent mutation
SNP	c14408t	N/A	1	100.0000%	ORF1ab (nsp12)	P323L
SNP	g15451a	N/A	1	100.0000%	ORF1ab (nsp12)	G671S
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	ΔLPP (amino acids 24-26), A27S
SNP	g21987a	N/A	1	100.0000%	Spike	G142D
SNP	a22001g	N/A	1	100.0000%	Spike	K147E
SNP	t22016c	N/A	1	100.0000%	Spike	W152R
SNP	c22033a	N/A	1	100.0000%	Spike	F157L
SNP	a22190g	N/A	1	100.0000%	Spike	I210V
SNP	t22200g	N/A	1	100.0000%	Spike	V213G
SNP	g22331a	N/A	1	100.0000%	Spike	G257S
SNP	g22577c	N/A	1	100.0000%	Spike	G339H
SNP	g22578a	N/A	1	100.0000%		
SNP	c22674t	N/A	1	100.0000%	Spike	S371F
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

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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	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	g22898a	N/A	1	100.0000%	Spike	G446S
SNP	t22942g	N/A	1	100.0000%	Spike	N460K
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	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	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	a24424t	N/A	1	100.0000%	Spike	Q954H
SNP	t24469a	N/A	1	100.0000%	Spike	N969K
SNP	c25000t	N/A	1	100.0000%	Spike	Silent mutation
SNP	c25416t	N/A	1	100.0000%	ORF3a	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	T9I
SNP	a26275g	N/A	1	100.0000%	Envelope	T11A
SNP	c26577g	N/A	1	100.0000%	Membrane	Q19E
SNP	g26709a	N/A	1	100.0000%	Membrane	A63T
SNP	c26858t	N/A	1	100.0000%	Membrane	Silent mutation
SNP	a27259c	N/A	1	100.0000%	ORF6	Silent mutation
SNP	g27382c	N/A	1	100.0000%	ORF6	D61L
SNP	a27383t	N/A	1	100.0000%		
SNP	t27384c	N/A	1	100.0000%		
SNP	c27807t	N/A	1	100.0000%	ORF7b	Silent mutation
SNP	a28271t	N/A	1	100.0000%	Intergenic (ORF8/ Nucleocapsid)	Untranslated
SNP	c28311t	N/A	1	100.0000%	Nucleocapsid	P13L
DEL	Δ28362-28370	N/A	-9	100.0000%	Nucleocapsid	ΔERS (amino acids 31-33)
SNP	g28881a	N/A	1	100.0000%	Nucleocapsid	R203K
SNP	g28882a	N/A	1	100.0000%		

Variant Type	Variant Position and Identified Alternative Base	Coverage ¹	Length of Variant	Frequency of Variant ¹	Gene (Region)	Amino Acid Mutation
SNP	g28883c	N/A	1	100.0000%	Nucleocapsid	G204R
SNP	a29510c	N/A	1	100.0000%	Nucleocapsid	S413R

¹Coverage 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.