

SARS-Related Coronavirus 2, Isolate USA/CA/VRLC012/2021 (Lineage P.2; Zeta Variant)

Catalog No. NR-55439

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate USA/CA/VRLC012/2021 was isolated from a mid-turbinate nasal swab from an adult on January 23, 2021 in California, USA. NR-55439 lot 70044002 was produced by infecting *Homo sapiens* lung adenocarcinoma epithelial cells (Calu-3; ATCC® HTB-55™) 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. Cell lysate and supernatant were clarified by centrifuging at 1500 × g for 10 minutes at 4°C.

Passage History:

VT(1)/C(1) (Johns Hopkins University/BEI Resources); VT = Vero E6 cells with Transmembrane Protease, Serine 2 Gene; C = Calu-3 cells

Lot: 70044002

Manufacturing Date: 03MAY2021

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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, Isolate USA/CA/VRLC012/2021 (GISAID: EPI_ISL_1364506)	99.97% identity with SARS-CoV-2, Isolate USA/CA/VRLC012/2021 (GISAID: EPI_ISL_1364506)
Titer by TCID ₅₀ Assay in Calu-3 Cells by Cytopathic Effect ¹ (5 days at 37°C with 5% CO ₂)	Report results	3.1 × 10 ⁷ 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 × 10⁶ per mL, 1.6 × 10⁶ per mL and 8.9 × 10⁷ 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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APPENDIX I: NGS Information for NR-55439 lot 70044002

Sequence analysis using SBC v2.0 pipeline and freebayes v1.3.1 variant caller resulted in the discovery of five SNPs when compared to the reference sequence from EPI_ISL_1364506 (see Table I below). Additionally, both the reference sequence EPI_ISL_1364506 and NR-55439 lot 70044002 contained eighteen SNPs when compared to GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) (see Table II below).

Table I: Variants with different nucleotides between NR-55439 lot 70044002 and reference sequence EPI_ISL_1364506

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	c12786t	3703	1	24.7907%	ORF1ab (nsp9)	T34I
SNP	t14679c	2124	1	13.0885%	ORF1ab (nsp12)	Silent mutation
SNP	t22114c	1133	1	5.5605%	Spike	Silent mutation
SNP	t25911c	2081	1	5.1418%	ORF3a	Silent mutation
SNP	c29686t	9218	1	99.9783%	3'UTR	Untranslated region

Table II: Variants with different nucleotides between NR-55439 lot 70044002 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	c100t	N/A	1	100.0000%	5'UTR	Untranslated region
SNP	c241t	N/A	1	100.0000%	5'UTR	Untranslated region
SNP	c3037t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	t3477c	N/A	1	100.0000%	ORF1ab (nsp3)	V253A
SNP	t3766c	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c5694t	N/A	1	100.0000%	ORF1ab (nsp3)	P992L
SNP	c12053t	N/A	1	100.0000%	ORF1ab (nsp7)	L71F
SNP	a12964g	N/A	1	100.0000%	ORF1ab (nsp9)	Silent mutation
SNP	c14408t	N/A	1	100.0000%	ORF1ab (nsp12)	P323L
SNP	a19578g	N/A	1	100.0000%	ORF1ab (nsp14)	Silent mutation
SNP	g23012a	N/A	1	100.0000%	Spike	E484K
SNP	a23403g	N/A	1	100.0000%	Spike	D614G
SNP	g25088t	N/A	1	100.0000%	Spike	V1176F
SNP	c28253t	N/A	1	100.0000%	ORF8	Silent mutation

Variant Type	Variant Position and Identified Alternative Base	Coverage ¹	Length of Variant	Frequency of Variant ¹	Gene (Region)	Amino Acid Mutation
SNP	g28881a	N/A	1	100.0000%	Nucleocapsid	R203K
SNP	g28882a	N/A	1	100.0000%		R203K
SNP	g28883c	N/A	1	100.0000%	Nucleocapsid	G204R
SNP	g28975t	N/A	1	100.0000%	Nucleocapsid	M234I

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 (EPI_ISL_1364506) 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.