

SARS-Related Coronavirus 2, Isolate USA/CA/VRLC014/2021 (Lineage B.1.429; Epsilon Variant)

Catalog No. NR-55309

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate USA/CA/VRLC014/2021 was isolated from a nasal turbinate swab from a 41-year-old male on January 12, 2021 in California, USA. NR-55309 lot 70043100 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.

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: 70043100

Manufacturing Date: 22MAR2021

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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/VRLC014/2021 (GISAID: EPI_ISL_1364488)	99.99% identity with SARS-CoV-2, isolate USA/CA/VRLC014/2021 (GISAID: EPI_ISL_1364488)
Titer by TCID₅₀ Assay in Calu-3 Cells by Cytopathic Effect¹ (6 days at 37°C with 5% CO ₂)	Report results	2.8 × 10 ⁶ TCID ₅₀ per mL
Sterility (21-day incubation) Harpo's HTYE broth, 37°C and 26°C, aerobic ² 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.

²Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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12 JUL 2021

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APPENDIX I: NGS Information for NR-55309 lot 70043100

Sequence analysis using SBC v2.0 pipeline and freebayes v1.3.1 variant caller resulted in the discovery of one SNP when compared to the reference sequence EPI_ISL_1364488 (see Table I below). Additionally, both the reference sequence EPI_ISL_1364488 and NR-55309 lot 70043100 contained twenty-nine SNPs when compared to GenBank MN908947.3 (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-55309 lot 70043100 and reference sequence EPI_ISL_1364488

Variant Type	Variant Position and Identified Alternative Base	Coverage	Quality	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	c10507t	4338	4849	1	5.6478%	ORF1ab (nsp5)	Silent mutation

Table II: Variants with different nucleotides between NR-55309 lot 70043100 and GenBank MN908947.3 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome)

Variant Type	Variant Position and Identified Alternative Base	Coverage	Quality	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	c241t	2162	49314	1	99.8150%	5'UTR	Untranslated region
SNP	c1059t	2542	49314	1	99.9607%	ORF1ab (nsp2)	T85I
SNP	c2395t	3647	49314	1	99.9177%	ORF1ab (nsp2)	Silent mutation
SNP	t2597c	2480	49314	1	100.0000%	ORF1ab (nsp2)	Silent mutation
SNP	c3037t	1943	49314	1	99.8456%	ORF1ab (nsp3)	Silent mutation
SNP	c8947t	2049	49314	1	99.7072%	ORF1ab (nsp4)	Silent mutation
SNP	c12100t	3098	49314	1	99.9677%	ORF1ab (nsp8)	Silent mutation
SNP	a12878g	2581	49314	1	100.0000%	ORF1ab (nsp9)	I65V
SNP	c14408t	1504	49314	1	98.7367%	ORF1ab (nsp12)	P323L
SNP	g17014t	2161	49314	1	99.8612%	ORF1ab (nsp13)	D260Y
SNP	g21600t	4866	49314	1	99.8767%	Spike	S13I
SNP	c21846t	2075	49314	1	99.9518%	Spike	T95I
SNP	g22018t	2346	49314	1	99.9574%	Spike	W152C
SNP	t22441c	3316	49314	1	99.8794%	Spike	Silent mutation
SNP	t22917g	2146	49314	1	99.9068%	Spike	L452R
SNP	t23155c	2152	49314	1	99.8606%	Spike	Silent mutation
SNP	a23403g	3922	49314	1	99.9490%	Spike	D614G
SNP	g23587c	4249	49314	1	99.8823%	Spike	Q675H
SNP	t24349c	2842	49314	1	100.0000%	Spike	Silent mutation
SNP	g25563t	2941	49314	1	100.0000%	ORF3a	Q57H
SNP	c26681t	4086	49314	1	99.8287%	Membrane protein	Silent mutation
SNP	g27890t	11808	49314	1	99.9068%	Intergenic (ORF7b/ORF8)	Untranslated region
SNP	g28191t	10536	49314	1	99.8481%	ORF8	V100L
SNP	a28272t	10260	49314	1	99.8343%	Intergenic (ORF8/ Nucleocapsid)	Untranslated region
SNP	c28887t	8890	49314	1	99.9325%	Nucleocapsid	T205I
SNP	g28975t	6995	49314	1	99.8999%	Nucleocapsid	M234I
SNP	c29362t	5560	49314	1	99.6763%	Nucleocapsid	Silent mutation
SNP	c29421t	9576	49314	1	99.8329%	Nucleocapsid	P383L
SNP	a29839g	996	513	1	5.3213%	3'UTR	Untranslated region