Certificate of Analysis for NR-55308

SARS-Related Coronavirus 2, Isolate USA/CA/VRLC009/2021 (Lineage B.1.427; Epsilon Variant)

Catalog No. NR-55308

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate USA/CA/VRLC009/2021 was isolated from a nasopharyngeal swab on January 13, 2021 in California, USA. NR-55308 lot 70043098 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% CO_2.

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: 70043098 Manufacturing Date: 15MAR2021

BEI Resources is committed to ensuring digital accessibility for people with disabilities. This Certificate of Analysis contains complex tables and may not be fully accessible. Please let us know if you encounter accessibility barriers and a fully accessible document will be provided: E-mail: Contact@BEIResources.org. We try to respond to feedback within 24 hours.

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/VRLC009/2021 (GISAID: EPI_ISL_1364499)	99.98% identity with SARS-CoV-2, Isolate USA/CA/VRLC009/2021 (GISAID: EPI_ISL_1364499)
Titer by $TCID_{50}$ Assay in Calu-3 Cells by Cytopathic Effect[1] (6 days at 37°C with 5% CO_2)	Report results	1.4×10^6 $TCID_{50}$ 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 (1.6×10^6 per mL, 8.9×10^5 per mL and 1.6×10^6 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.

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

/Heather Couch/
Heather Couch 06 JUL 2021
Program 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.

Support Provided by NIAID

APPENDIX I: NGS Information for NR-55308 lot 70043098

Sequence analysis using SBC v2.0 pipeline and freebayes v1.3.1 variant caller resulted in the discovery of three SNPs and three ambiguous variants (AMB) when compared to the reference sequence from GISAID EPI_ISL_1364499 (see Table I below). The given reference sequence GISAID EPI_ISL_1364499 is 141 base pairs (bp) shorter (1 bp upstream, 140 bp downstream) than the GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) sequence and had four ambiguous bases having a nucleotide among Y, R, W, K, M, S, B, D, H, V, or N in the sequence. There are three variants referred to as AMB in which the nucleotide in NR-55308 lot 70043098 and Wuhan-Hu-1 sequence (GenBank MN908947) are known but the reference sequence EPI_ISL_1364499 is ambiguous at that position (see Table I below). Due to the ambiguity of the reference sequence at this position, the variant type is otherwise unlikely to be determined, including whether there is any variation at all (i.e. the ambiguous nucleotide in the strain reference sequence GISAID EPI_ISL_1364499 may be identical to both NR-55308 lot 70043098 and the Wuhan-Hu-1 sequence GenBank MN908947). Additionally, both the reference sequence EPI_ISL_1364499 and NR-55308 lot 70043098 contained twenty-four SNPs when compared to GenBank MN908947 (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-55308 lot 70043098 and reference sequence EPI_ISL_1364499

Variant Type	Variant Position and Identified Alternative Base	Coverage	Quality	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
AMB	g7925r_g[1]	6794	215551	1	99.9411%	ORF1ab (nsp3)	Silent mutation
SNP	t15531c	7734	7883	1	5.5340%	ORF1ab (nsp12)	Silent mutation
SNP	t22114c	5159	3611	1	6.4160%	Spike	Silent mutation
SNP	a25201g	4420	2820	1	6.0181%	Spike	Silent mutation
AMB	t29709w_t	24753	818815	1	99.9636%	3'UTR	Untranslated region
AMB	g29711s_g	24753	818815	1	99.9636%	3'UTR	Untranslated region

[1]g7925r_g represents the original nucleotide of 'g' in GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) and the ambiguous nucleotide 'r' in reference sequence GISAID EPI_ISL_1364499. _g represents the nucleotide 'g' observed in NR-55308 lot 70043098.

Table II: Variants with different nucleotides between NR-55308 lot 70043098 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	5798	49314	1	99.8965%	5'UTR	Untranslated region
SNP	c1059t	7267	49314	1	99.9312%	ORF1ab (nsp2)	T85I
SNP	c3037t	6177	49314	1	99.8543%	ORF1ab (nsp3)	Silent mutation
SNP	g3231t	3521	49314	1	99.8580%	ORF1ab (nsp3)	G171V
SNP	c3817t	3943	49314	1	99.8732%	ORF1ab (nsp3)	Silent mutation
SNP	c6317a	16835	49314	1	99.8218%	ORF1ab (nsp3)	P1200T
SNP	g9738c	4445	49314	1	99.7525%	ORF1ab (nsp4)	S395T
SNP	c13019t	16083	49314	1	99.8756%	ORF1ab (nsp9)	Silent mutation
SNP	g13713a	10596	49314	1	99.7641%	ORF1ab (nsp12)	Silent mutation
SNP	c14408t	4537	49314	1	98.3910%	ORF1ab (nsp12)	P323L

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

Variant Type	Variant Position and Identified Alternative Base	Coverage	Quality	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	c16394t	7664	49314	1	99.8434%	ORF1ab (nsp13)	P53L
SNP	g17014t	5581	49314	1	99.5341%	ORF1ab (nsp13)	D260Y
SNP	g21600t	14390	49314	1	99.8054%	Spike	S13I
SNP	g22018t	6785	49314	1	99.9263%	Spike	W152C
SNP	g22335t	8873	49314	1	99.7633%	Spike	W258L
SNP	c22597t	8727	49314	1	99.0375%	Spike	Silent mutation
SNP	t22917g	5845	49314	1	99.7776%	Spike	L452R
SNP	a23403g	14299	49314	1	99.9650%	Spike	D614G
SNP	g25563t	7994	49314	1	99.8249%	ORF3a	Q57H
SNP	c26681t	13021	49314	1	99.7850%	Membrane protein	Silent mutation
SNP	c28087t	12367	49314	1	99.3531%	ORF8	A65V
SNP	a28272t	44019	49314	1	99.8955%	Intergenic (ORF8/Nucleocapsid)	Untranslated region
SNP	c28887t	17557	49314	1	99.9146%	Nucleocapsid	T205I
SNP	c29362t	21543	49314	1	99.6333%	Nucleocapsid	Silent mutation

BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898