Certificate of Analysis for NR-55611

SARS-Related Coronavirus 2, Isolate hCoV-19/USA/PHC658/2021 (Lineage B.1.617.2; Delta Variant)

Catalog No. NR-55611

Product Description:

Based on preliminary *in vivo* data, investigators should exercise caution when propagating NR-55611 (hCoV-19/USA/PHC658/2021) and/or using for animal exposures. Sequence data of the clinical sample shows a deletion within ORF7a (aa-44-100) which may have the potential to impact virulence. Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/USA/PHC658/2021 was isolated in Memphis, Tennessee, USA in May 2021. NR-55611 lot 70045238 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:

VTA2(1)VT(1)/C(1) (St. Jude Children's Research Hospital/BEI Resources); VTA2 = *Cercopithecus aethiops* kidney cells with transmembrane protease, serine 2 gene (TMPRSS2) and angiotensin-converting enzyme 2 (ACE2); VT = *Cercopithecus aethiops* kidney cells with TMPRSS2; C = Calu-3

Lot: 70045238 Manufacturing Date: 07JUN2021

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, hCoV-19/USA/PHC658/2021 depositor sequence	99.99% identity with SARS-CoV-2, hCoV-19/USA/PHC658/2021 depositor sequence
Titer by $TCID_{50}$ Assay in Calu-3 Cells by Cytopathic Effect[1] (5 days at 37°C and 5% CO_2)	Report results	6.5×10^5 $TCID_{50}$ 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 (1.6×10^5 per mL, 8.9×10^5 per mL and 8.9×10^5 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 03 AUG 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-55611 lot 70045238

Sequence analysis using SBC v2.0 pipeline and freebayes v1.3.1 variant caller resulted in the discovery of three SNPs when compared to the reference sequence from the depositor (see Table I below). Additionally, both the reference sequence and NR-55611 lot 70045238 contained thirty SNPs and three deletions (DEL) when compared to GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) (see Table II below). The 173-nucleotide deletion in open reading frame 7a (ORF7a) introduces a frameshift extending the protein into open reading frame 7b (ORF7b). This deletion is present in both the provided reference sequence and NR-55611 lot 70045238 as compared to GenBank MN908947.

Table I: Variants with different nucleotides between NR-55611 lot 70045238 and reference sequence from the depositor

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	g21987a	1231	1	99.5126%	Spike	G142D
SNP	c28698a	1794	1	91.5273%	Nucleocapsid	P142Q
SNP	a29839g	428	1	5.3738%	3'UTR	Untranslated region

Table II: Variants with different nucleotides between NR-55611 lot 70045238 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 region
SNP	c241t	N/A	1	100.0000%	5'UTR	Untranslated region
SNP	g1055a	N/A	1	100.0000%	ORF1ab (nsp2)	D84N
SNP	c1191t	N/A	1	100.0000%	ORF1ab (nsp2)	P129L
SNP	c1267t	N/A	1	100.0000%	ORF1ab (nsp2)	Silent mutation
SNP	c3037t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c5184t	N/A	1	100.0000%	ORF1ab (nsp3)	P822L
SNP	g9203a	N/A	1	100.0000%	ORF1ab (nsp4)	D217N
SNP	t9678c	N/A	1	100.0000%	ORF1ab (nsp4)	F375S
SNP	c11005a	N/A	1	100.0000%	ORF1ab (nsp6)	H11Q
SNP	c14408t	N/A	1	100.0000%	ORF1ab (nsp12)	P323L
SNP	a17496g	N/A	1	100.0000%	ORF1ab (nsp13)	Silent mutation
SNP	a20396g	N/A	1	100.0000%	ORF1ab (nsp15)	K259R
SNP	c21618g	N/A	1	100.0000%	Spike	T19R
SNP	a21792c	N/A	1	100.0000%	Spike	K77T

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[1]	Length of Variant	Frequency of Variant[1]	Gene (Region)	Amino Acid Mutation
DEL	Δ22029-22034	N/A	-6	100.0000%	Spike	ΔFR (amino acids 157-158)
SNP	t22917g	N/A	1	100.0000%	Spike	L452R
SNP	c22995a	N/A	1	100.0000%	Spike	T478K
SNP	a23403g	N/A	1	100.0000%	Spike	D614G
SNP	c23604g	N/A	1	100.0000%	Spike	P681R
SNP	g24410a	N/A	1	100.0000%	Spike	D950N
SNP	c25469t	N/A	1	100.0000%	ORF3a	S26L
SNP	t26767c	N/A	1	100.0000%	Membrane protein	I82T
DEL	Δ27522-27694	N/A	-173	100.0000%	ORF7a	Δ(amino acids 44-100)fs.ext*41[2]
SNP	c27752t	N/A	1	100.0000%	ORF7a	T120I
SNP	g28198t	N/A	1	100.0000%	ORF8	C102F
SNP	c28253t	N/A	1	100.0000%	ORF8	Silent mutation
DEL	Δ28271	N/A	-1	100.0000%	Intergenic (ORF8/ Nucleocapsid)	Untranslated region
SNP	a28461g	N/A	1	100.0000%	Nucleocapsid	D63G
SNP	g28881t	N/A	1	100.0000%	Nucleocapsid	R203M
SNP	g29402t	N/A	1	100.0000%	Nucleocapsid	D377Y
SNP	t29512c	N/A	1	100.0000%	Nucleocapsid	Silent mutation
SNP	g29742t	N/A	1	100.0000%	3'UTR	Untranslated region

[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.

[2]Δ(amino acids 44-100)fs.ext*41: The 173-nucleotide deletion results in the deletion of a large portion of open reading frame 7a (ORF7a) from amino acids 44-100, as well as a frameshift (fs) that also results in an extension of the protein with the new stop codon (*) located 41 amino acids downstream of the original stop codon at the end of open reading frame 7b (ORF7b).

BEI Resources
www.beiresources.org

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