Certificate of Analysis for NR-55705

SARS-Related Coronavirus 2, Isolate hCoV-19/USA/MD-HP03056/2021 (Lineage B.1.525; Eta Variant)

Catalog No. NR-55705

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/USA/MD-HP03056/2021 was isolated from an older human in Maryland, USA, on March 4, 2021. NR-55705 lot 70047233 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:

VT(1)/C(1) (Johns Hopkins University/BEI Resources); VT = *Cercopithecus aethiops* kidney cells with transmembrane protease, serine 2 gene (TMPRSS2); C = Calu-3

Lot: 70047233 Manufacturing Date: 02SEP2021

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/MD-HP03056/2021 (GISAID: EPI_ISL_1405588)	99.99% identity with SARS-CoV-2, hCoV-19/USA/MD-HP03056/2021 (GISAID: EPI_ISL_1405588)
Titer by $TCID_{50}$ Assay in Calu-3 Cells by Cytopathic Effect[1] (7 days at 37°C and 5% CO_2)	Report results	2.0×10^6 $TCID_{50}$ 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, 1.6×10^6 per mL and 2.8×10^6 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 28 OCT 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-55705 lot 70047233

Sequence analysis using SBC v2.0 pipeline and freebayes v1.3.1 variant caller resulted in the discovery of two SNPs when compared to the reference sequence from GISAID EPI_ISL_1405588 (see Table I below). Additionally, both the reference sequence EPI_ISL_1405588 and NR-55705 lot 70047233 contained twenty-eight SNPs and five deletions (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-55705 lot 70047233 and reference sequence EPI_ISL_1405588

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	a11160g	1562	1	99.8720%	ORF1ab (nsp6)	K63R
SNP	t14679c	1206	1	5.8872%	ORF1ab (nsp12)	Silent mutation

Table II: Variants with different nucleotides between NR-55705 lot 70047233 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	c1498t	N/A	1	100.0000%	ORF1ab (nsp2)	Silent mutation
SNP	a1807g	N/A	1	100.0000%	ORF1ab (nsp2)	Silent mutation
SNP	c1887t	N/A	1	100.0000%	ORF1ab (nsp2)	A361V
SNP	g2659a	N/A	1	100.0000%	ORF1ab (nsp2)	Silent mutation
SNP	c3037t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c5869t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c6285t	N/A	1	100.0000%	ORF1ab (nsp3)	T1189I
SNP	a8031g	N/A	1	100.0000%	ORF1ab (nsp3)	K1771R
SNP	t8323c	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	t8593c	N/A	1	100.0000%	ORF1ab (nsp4)	Silent mutation
SNP	c9565t	N/A	1	100.0000%	ORF1ab (nsp4)	Silent mutation
DEL	Δ11288-11296	N/A	-9	100.0000%	ORF1ab (nsp6)	ΔSGF (amino acids 106-108)
SNP	c14407t	N/A	1	100.0000%	ORF1ab (nsp12)	P323F
SNP	c14408t	N/A	1	100.0000%		

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
SNP	c18171t	N/A	1	100.0000%	ORF1ab (nsp14)	Silent mutation
SNP	a20724g	N/A	1	100.0000%	ORF1ab (nsp16)	Silent mutation
SNP	c21762t	N/A	1	100.0000%	Spike	A67V
DEL	Δ21765-21770	N/A	-6	100.0000%	Spike	ΔHV (amino acid 69-70)
DEL	Δ21991-21993	N/A	-3	100.0000%	Spike	ΔY144
SNP	g23012a	N/A	1	100.0000%	Spike	E484K
SNP	a23403g	N/A	1	100.0000%	Spike	D614G
SNP	g23593c	N/A	1	100.0000%	Spike	Q677H
SNP	t24224c	N/A	1	100.0000%	Spike	F888L
SNP	c24748t	N/A	1	100.0000%	Spike	Silent mutation
SNP	c26305t	N/A	1	100.0000%	Envelope	L21F
SNP	t26767c	N/A	1	100.0000%	Membrane	I82T
DEL	Δ27205-27207	N/A	-3	100.0000%	ORF6	ΔF (amino acid 2)
DEL	Δ28278-28280	N/A	-3	100.0000%	Nucleocapsid	S2Y, ΔD (amino acid 3)
SNP	c28308g	N/A	1	100.0000%	Nucleocapsid	A12G
SNP	a28699g	N/A	1	100.0000%	Nucleocapsid	Silent mutation
SNP	c28887t	N/A	1	100.0000%	Nucleocapsid	T205I
SNP	g29543t	N/A	1	100.0000%	Intergenic (Nucleocapsid/ ORF10)	Untranslated

[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.

BEI Resources
www.beiresources.org

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