

**SARS-Related Coronavirus 2, Isolate hCoV-19/USA/MD-HP05647/2021 (Lineage B.1.617.2; Delta variant)**

**Catalog No. NR-55672**

**Product Description:**

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/USA/MD-HP05647/2021 was isolated in Maryland, USA on April 27, 2021. NR-55672 lot 70046635 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<sub>2</sub>. 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 (Vero E6-TMPRSS2); C = Calu-3

**Lot: 70046635**

**Manufacturing Date: 02AUG2021**

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](mailto:Contact@BEIResources.org). We try to respond to feedback within 24 hours.

TEST	SPECIFICATIONS	RESULTS
<b>Identification by Infectivity in Calu-3 Cells</b>	Cell rounding and detachment	Cell rounding and detachment
<b>Next-Generation Sequencing (NGS) of Complete Genome Using Illumina® iSeq™ 100 Platform</b> (Refer to Appendix I for NGS information)	≥ 98% identity with SARS-CoV-2, hCoV-19/USA/MD-HP05647/2021 (GISAID: EPI_ISL_2331496)	99.99% identity with SARS-CoV-2, hCoV-19/USA/MD-HP05647/2021 (GISAID: EPI_ISL_2331496)
<b>Titer by TCID<sub>50</sub> Assay in Calu-3 Cells by Cytopathic Effect<sup>1</sup></b> (6 days at 37°C and 5% CO <sub>2</sub> )	Report results	1.8 × 10 <sup>7</sup> TCID <sub>50</sub> per mL <sup>2</sup>
<b>Sterility (21-day incubation)</b> Harpo's HTYE broth, 37°C and 26°C, aerobic <sup>3</sup> 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
<b>Mycoplasma Contamination</b> 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

<sup>1</sup>The Tissue Culture Infectious Dose 50% (TCID<sub>50</sub>) endpoint is the 50% infectious endpoint in cell culture. The TCID<sub>50</sub> 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<sub>50</sub>) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID<sub>50</sub> provides a measure of the titer (or infectivity) of a virus preparation.

<sup>2</sup>Titer was determined by cytopathic effects (CPE) and completed in triplicate (1.6 × 10<sup>7</sup> per mL, 2.8 × 10<sup>7</sup> per mL and 8.9 × 10<sup>6</sup> per mL). The average of the three values is reported.

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

/Heather Couch/  
Heather Couch

07 SEP 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.

ATCC® is a trademark of the American Type Culture Collection.

You are authorized to use this product for research use only. It is not intended for human use.



**APPENDIX I: NGS Information for NR-55672 lot 70046635**

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-55672 lot 70046635 contained thirty-six SNPs and three deletions (DEL) 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-55672 lot 70046635 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	c15960t	4160	1	9.1587%	ORF1ab (nsp12)	Silent mutation
SNP	c19269t	2459	1	99.8373%	ORF1ab (nsp14)	Silent mutation
SNP	c26681t	5659	1	5.8491%	Membrane	Silent mutation

**Table II: Variants with different nucleotides between NR-55672 lot 70046635 and GenBank MN908947.3 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome)**

Variant Type	Variant Position and Identified Alternative Base	Coverage <sup>1</sup>	Length of Variant	Frequency of Variant <sup>1</sup>	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	c3037t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	g4181t	N/A	1	100.0000%	ORF1ab (nsp3)	A488S
SNP	c6402t	N/A	1	100.0000%	ORF1ab (nsp3)	P1228L
SNP	c7124t	N/A	1	100.0000%	ORF1ab (nsp3)	P1469S
SNP	c8986t	N/A	1	100.0000%	ORF1ab (nsp4)	Silent mutation
SNP	g9053t	N/A	1	100.0000%	ORF1ab (nsp4)	V167L
SNP	c9811t	N/A	1	100.0000%	ORF1ab (nsp4)	Silent mutation
SNP	c10029t	N/A	1	100.0000%	ORF1ab (nsp4)	T492I
SNP	a11201g	N/A	1	100.0000%	ORF1ab (nsp6)	T77A
SNP	a11332g	N/A	1	100.0000%	ORF1ab (nsp6)	Silent mutation
SNP	c14408t	N/A	1	100.0000%	ORF1ab (nsp12)	P323L
SNP	g15451a	N/A	1	100.0000%	ORF1ab (nsp12)	G671S
SNP	c16466t	N/A	1	100.0000%	ORF1ab (nsp13)	P77L
SNP	c19220t	N/A	1	100.0000%	ORF1ab (nsp14)	A394V

Variant Type	Variant Position and Identified Alternative Base	Coverage <sup>1</sup>	Length of Variant	Frequency of Variant <sup>1</sup>	Gene (Region)	Amino Acid Mutation
SNP	c21618g	N/A	1	100.0000%	Spike	T19R
SNP	a22029g	N/A	1	100.0000%	Spike	E156G
SNP	g22030a	N/A	1	100.0000%		
DEL	Δ22029-22034	N/A	-6	100.0000%	Spike	ΔFR (AA157-158)
SNP	c22326t	N/A	1	100.0000%	Spike	S255F
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	I82T
SNP	g26918t	N/A	1	100.0000%	Membrane	Silent mutation
SNP	t27638c	N/A	-1	100.0000%	ORF7a	V82A
SNP	c27752t	N/A	1	100.0000%	ORF7a	T120I
SNP	c27874t	N/A	1	100.0000%	ORF7b	T40I
DEL	Δ28248-28253	N/A	-6	100.0000%	ORF8	ΔDF (AA119-120)
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	g28916t	N/A	1	100.0000%	Nucleocapsid	G215C
SNP	g29402t	N/A	1	100.0000%	Nucleocapsid	D377Y
SNP	g29742t	N/A	1	100.0000%	3'UTR	Untranslated region

<sup>1</sup>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.