SUPPORTING INFECTIOUS DISEASE RESEARCH

# SARS-Related Coronavirus 2, Isolate hCoV-19/USA/MD-HP01542/2021 (Lineage B.1.351), in *Homo sapiens* Lung Adenocarcinoma (Calu-3) Cells

# Catalog No. NR-55282

## **Product Description:**

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/USA/MD-HP01542/2021 was isolated from a nasal swab from a human patient in January 2021 in Maryland, USA. NR-55282 was produced by infecting *Homo sapiens* lung adenocarcinoma cells (Calu-3; ATCC<sup>®</sup> HTB-55<sup>™</sup>) with SARS-CoV-2, isolate hCoV-19/USA/MD-HP01542/2021 and incubating in Eagle's Minimum Essential Medium (ATCC<sup>®</sup> 30-2003<sup>™</sup>) supplemented with 2% fetal bovine serum (ATCC<sup>®</sup> 30-2020<sup>™</sup>) for 3 days at 37°C with 5% CO<sub>2</sub>. Cell lysate and supernatant was clarified by centrifugation and vialed.

### Passage History:

VT(1)/C(1) (John Hopkins University/BEI Resources); VT = *Cercopithecus aethiops* kidney cells with transmembrane protease, serine 2 gene (Vero E6/TMPRSS2); C = Calu-3 cells

# Lot: 70043066

# Manufacturing Date: 12MAR2021

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: <u>Contact@BEIResources.org</u>. 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 <sup>®</sup> iSeq™ 100 Platform (Refer to Appendix I for NGS information)	≥ 98% identity with SARS- CoV-2, hCoV-19/USA/MD- HP01542/2021 (GISAID: EPI_ISL_890360)	99.97% identity with SARS- CoV-2, hCoV-19/USA/MD- HP01542/2021 (GISAID: EPI_ISL_890360)	
Titer by TCID₅ Assay in Calu-3 Cells by Cytopathic Effect <sup>1</sup> (5 days at 37°C and 5% CO₂)	Report results	$1.5 \times 10^5 \text{ TCID}_{50} \text{ per mL}^2$	
Sterility (21-day incubation)			
Harpo's HTYE broth, 37°C and 26°C, aerobic <sup>3</sup>	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	

<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 (2.8 × 10<sup>5</sup> per mL, 8.9 × 10<sup>4</sup> per mL and 8.9 × 10<sup>4</sup> per mL). The average of the three values is reported.

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

**b**|**e**|**i** resources

SUPPORTING INFECTIOUS DISEASE RESEARCH

## /Heather Couch/ Heather Couch

#### 03 MAY 2021

#### Program Manager or designee, ATCC Federal Solutions

ATCC<sup>®</sup>, 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<sup>®</sup>'s knowledge.

ATCC<sup>®</sup> 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-55282 lot 70043066

Sequence analysis using SBC v2.0 pipeline and freebayes v1.3.1 variant caller resulted in the discovery of five SNPs when compared to the reference sequence from EPI\_ISL\_890360 (see Table I below). Additionally, both the reference sequence EPI\_ISL\_890360 and NR-55282 lot 70043066 contained twenty-five SNPs and two deletions (Indel) 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-55282 lot 70043066 and reference sequence EPI\_ISL\_890360

Variant Type	Variant Position and Identified Alternative Base	Coverage	Quality	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	g10533t	1224	945	1	5.2288%	ORF1ab (nsp5)	C160F
SNP	c11750t	1291	31304	1	75.6003%	ORF1ab (nsp6)	L260F
SNP	c13164t	751	1588	1	10.6242%	ORF1ab (nsp10)	T47I
SNP	t14679c	595	490	1	7.5080%	ORF1ab (nsp12)	Silent mutation
SNP	a25201g	456	501	1	8.9520%	Spike	Silent mutation

# Table II: Variants with different nucleotides between NR-55282 lot 70043066 and GenBank MN908947 (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	g174t	951	32321	1	97.5916%	5' UTR	Untranslated region
SNP	t201c	590	19134	1	98.1387%	5' UTR	Untranslated region
SNP	c241t	576	20121	1	100.0000%	5' UTR	Untranslated region
SNP	c1059t	718	25101	1	98.6072%	ORF1ab (nsp2)	T85I
SNP	a2692t	476	16285	1	97.0588%	ORF1ab (nsp2)	Silent mutation
SNP	c3037t	629	22342	1	99.8413%	ORF1ab (nsp3)	Silent mutation
SNP	g5230t	1079	37617	1	97.8723%	ORF1ab (nsp3)	K837N
SNP	a10323g	1004	33538	1	97.8088%	ORF1ab (nsp5)	K90R
Indel	11288_11296del	613	18978	-9	99.8371%	ORF1ab (nsp6)	ΔSGF (aa 106-108)
SNP	c14408t	441	15965	1	93.2347%	ORF1ab (nsp12)	P323L
SNP	g18090a	1383	47056	1	97.8308%	ORF1ab (nsp14)	Silent mutation
SNP	c21614t	1417	49314	1	99.5074%	Spike	L18F
SNP	a21801c	720	24256	1	99.3056%	Spike	D80A
SNP	a22206g	715	22995	1	98.4637%	Spike	D215G
Indel	22286_22294del	799	25046.4	-9	99.0087%	Spike	ΔLAL (aa 242-244)
SNP	g22813t	469	16413	1	98.0810%	Spike	K417N

BEI Resources www.beiresources.org E-mail: <u>contact@beiresources.org</u> Tel: 800-359-7370 Fax: 703-365-2898 **b**|**e**|**i** resources

# **Certificate of Analysis for NR-55282**

SUPPORTING INFECTIOUS DISEASE RESEARCH

Variant Type	Variant Position and Identified Alternative Base	Coverage	Quality	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	g23012a	840	30569	1	99.6429%	Spike	E484K
SNP	a23063t	857	31489	1	99.4166%	Spike	N501Y
SNP	a23403g	931	33463	1	100.0000%	Spike	D614G
SNP	c23664t	960	32597	1	98.2310%	Spike	A701V
SNP	g25563t	834	28786	1	98.4431%	ORF3a	Q57H
SNP	c25904t	971	35229	1	98.8671%	ORF3a	S171L
SNP	g25906t	971	34836	1	98.8671%	ORF3a	Silent mutation
SNP	c26456t	2170	49314	1	99.3088%	Envelope	P71L
SNP	c28253t	2573	49314	1	97.6511%	ORF8	Silent mutation
SNP	c28887t	1411	49314	1	99.1507%	Nucleocapsid	T205I
SNP	a29839g	366	263	1	7.0081%	3' UTR	Untranslated region