Certificate of Analysis for NR-55483

SARS-Related Coronavirus 2, Isolate hCoV-19/USA/PHC205/2021 (Lineage B.1.1.519)

Catalog No. NR-55483

Product Description:

Note: The label for lot 70047320 indicates the lineage is B.1.519. The correct lineage is B.1.1.519. Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/USA/PHC205/2021 (also referred to as hCoV-19/USA/unPHC205/2021) was isolated from a nasopharyngeal swab in Memphis, Tennessee, USA on May 22, 2021. NR-55483 lot 70047320 was produced by infecting *Homo sapiens* lung adenocarcinoma 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:

VT2A2(1), VT2(1)/C(1) (St. Jude Children's Research Hospital/BEI Resources); VT2A2 = Vero E6 cells with transmembrane protease, serine 2 (TMPRSS2) gene and ACE2; VT2 = Vero E6 cells with TMPRSS2 gene ; C = Calu-3 cells

Lot: 70047320 Manufacturing Date: 03SEP2021

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/PHC205/2021 (GenBank: MZ358923.1)	100% identity with SARS-CoV-2, hCoV-19/USA/PHC205/2021 (GenBank: MZ358923.1)
Titer by $TCID_{50}$ Assay in Calu-3 Cells by Cytopathic Effect[1] (7 days at 37°C and 5% CO_2)	Report results	4.4×10^6 $TCID_{50}$ 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, 2.8×10^6 per mL and 8.9×10^6 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 27 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-55483 lot 70047320

Sequence analysis using SBC v2.0 pipeline and freebayes v1.3.1 variant caller resulted in the discovery of one SNP when compared to the reference sequence GenBank MZ358923.1 (see Table I below). Additionally, both the reference sequence MZ358923.1 and NR-55483 lot 70047320 contained twenty-nine SNPs 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-55483 lot 70047320 and reference sequence MZ358923.1

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	a29871g	159	1	21.3836%	3'UTR	Untranslated region

Table II: Variants with different nucleotides between NR-55483 lot 70047320 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	c203t	N/A	1	100.0000%	5'UTR	Untranslated region
SNP	c222t	N/A	1	100.0000%	5'UTR	Untranslated region
SNP	c241t	N/A	1	100.0000%	5'UTR	Untranslated region
SNP	a1740g	N/A	1	100.0000%	ORF1ab (nsp2)	K312R
SNP	c3037t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c3140t	N/A	1	100.0000%	ORF1ab (nsp3)	P141S
SNP	g3351a	N/A	1	100.0000%	ORF1ab (nsp3)	S211N
SNP	a6704t	N/A	1	100.0000%	ORF1ab (nsp3)	N1329Y
SNP	c10029t	N/A	1	100.0000%	ORF1ab (nsp4)	T492I
SNP	c10954t	N/A	1	100.0000%	ORF1ab (nsp5)	Silent mutation
SNP	a11117g	N/A	1	100.0000%	ORF1ab (nsp6)	I49V
SNP	c12789t	N/A	1	100.0000%	ORF1ab (nsp9)	T35I
SNP	c14408t	N/A	1	100.0000%	ORF1ab (nsp12)	P323L
SNP	g16943t	N/A	1	100.0000%	ORF1ab (nsp13)	S236I
SNP	c18129t	N/A	1	100.0000%	ORF1ab (nsp14)	Silent mutation
SNP	t19839c	N/A	1	100.0000%	ORF1ab (nsp15)	Silent mutation
SNP	c21306t	N/A	1	100.0000%	ORF1ab (nsp16)	Silent mutation
SNP	c22995a	N/A	1	100.0000%	Spike	T478K
SNP	a23403g	N/A	1	100.0000%	Spike	D614G

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	c23604a	N/A	1	100.0000%	Spike	P681H
SNP	a23756g	N/A	1	100.0000%	Spike	T732A
SNP	t26561c	N/A	1	100.0000%	Membrane	Silent Mutation
SNP	c26735t	N/A	1	100.0000%	Membrane	Silent Mutation
SNP	c26885t	N/A	1	100.0000%	Membrane	Silent Mutation
SNP	g28881a	N/A	1	100.0000%	Nucleocapsid	R203K
SNP	g28882a	N/A	1	100.0000%		
SNP	g28883c	N/A	1	100.0000%	Nucleocapsid	G204R
SNP	c29197t	N/A	1	100.0000%	Nucleocapsid	Silent Mutation
SNP	g29527t	N/A	1	100.0000%	Nucleocapsid	Q418H

[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