Certificate of Analysis for NR-55331

SARS-Related Coronavirus 2, Isolate hCoV-19/USA/CA-CDC-48018/2020 (Lineage B.1.427)

Catalog No. NR-55331

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/USA/CA-CDC-48018/2020 was isolated from nasopharyngeal aspirate of a 65-year-old female in California, USA on December 10, 2020. NR-55331 lot 70043181 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:

V(2)/C(1) (Centers for Disease Control and Prevention/BEI Resources); V = *Cercopithecus aethiops* kidney cells (Vero); C = Calu-3 cells

Lot: 70043181 Manufacturing Date: 22MAR2021

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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/CA-CDC-48018/2020	99.99% identity with SARS-CoV-2, hCoV-19/USA/CA-CDC-48018/2020
Titer by $TCID_{50}$ Assay in Calu-3 Cells by Cytopathic Effect[1] (6 days at 37°C and 5% CO_2)	Report results	8.9×10^6 $TCID_{50}$ per mL
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic[2]	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]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

/Heather Couch/
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Program Manager or designee, ATCC Federal Solutions

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APPENDIX I: NGS Information for NR-55331 lot 70043181

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 GISAID EPI_ISL_1225965 (see Table I below). Additionally, both the reference sequence EPI_ISL_1225965 and NR-55331 lot 70043181 contained twenty-five SNPs 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-55331 lot 70043181 and reference sequence EPI_ISL_1225965

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	a9852g	3695	1	23.7618%	ORF1ab (nsp4)	D433G
SNP	c11750t	7055	1	10.7725%	ORF1 (nsp6)	L260F
SNP	a29839g	1508	1	5.9682%	3'UTR	Untranslated region

Table II: Variants with different nucleotides between NR-55331 lot 70043181 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 region
SNP	c1059t	N/A	1	100.0000%	ORF1ab (nsp2)	T85I
SNP	c3037t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c3817t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c8895t	N/A	1	100.0000%	ORF1ab (nsp4)	T114I
SNP	g9738c	N/A	1	100.0000%	ORF1ab (nsp4)	S395T
SNP	g10300t	N/A	1	100.0000%	ORF1ab (nsp5)	M82I
SNP	c13019t	N/A	1	100.0000%	ORF1ab (nsp9)	Silent mutation
SNP	g13713a	N/A	1	100.0000%	ORF1ab (nsp12)	Silent mutation
SNP	c14408t	N/A	1	100.0000%	ORF1ab (nsp12)	P323L
SNP	c16394t	N/A	1	100.0000%	ORF1ab (nsp13)	P53L
SNP	g17014t	N/A	1	100.0000%	ORF1ab (nsp13)	D260Y
SNP	g21600t	N/A	1	100.0000%	Spike	S13I
SNP	g22018t	N/A	1	100.0000%	Spike	W152C
SNP	g22335t	N/A	1	100.0000%	Spike	W258L
SNP	c22597t	N/A	1	100.0000%	Spike	Silent mutation
SNP	t22917g	N/A	1	100.0000%	Spike	L452R
SNP	a23403g	N/A	1	100.0000%	Spike	D614G
SNP	g25563t	N/A	1	100.0000%	ORF3a	Q57H
SNP	c26681t	N/A	1	100.0000%	Membrane protein	Silent mutation
SNP	c27213t	N/A	1	100.0000%	ORF6	Silent mutation
SNP	c28087t	N/A	1	100.0000%	ORF8	A65V
SNP	a28272t	N/A	1	100.0000%	Intergenic (ORF8/ Nucleocapsid)	Untranslated region
SNP	c28887t	N/A	1	100.0000%	Nucleocapsid	T205I

Variant Type	Variant Position and Identified Alternative Base	Coverage[1]	Length of Variant	Frequency of Variant[1]	Gene (Region)	Amino Acid Mutation
SNP	c29362t	N/A	1	100.0000%	Nucleocapsid	Silent mutation

[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.

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