

SARS-Related Coronavirus 2, Isolate hCoV-19/USA/CA-CDC-50070/2020 (Lineage B.1.429)

Catalog No. NR-55332

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/USA/CA-CDC-50070/2020 was isolated from a nasopharyngeal aspirate in California, USA on December 23, 2020. NR-55332 lot 70043179 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% CO₂. 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; C = Calu-3 cells

Lot: 70043179

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-50070/2020 (GISAID: EPI_ISL_1225966)	100% identity with SARS-CoV-2, hCoV-19/USA/CA-CDC-50070/2020 (GISAID: EPI_ISL_1225966)
Titer by TCID₅₀ Assay in Calu-3 Cells by Cytopathic Effect¹ (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 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
Mycoplasma Contamination 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

¹The Tissue Culture Infectious Dose 50% (TCID₅₀) endpoint is the 50% infectious endpoint in cell culture. The TCID₅₀ 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% (LD₅₀) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID₅₀ provides a measure of the titer (or infectivity) of a virus preparation.

²Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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APPENDIX I: NGS Information for NR-55332 lot 70043179

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 from GISAID EPI_ISL_1225966 (see Table I below). Additionally, both the reference sequence EPI_ISL_1225966 and NR-55332 lot 70043179 contained twenty-six 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-55332 lot 70043179 and reference sequence EPI_ISL_1225966

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	a25201g	1287	1	5.3613%	Spike	Silent mutation

Table II: Variants with different nucleotides between NR-55332 lot 70043179 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	c1059t	N/A	1	100.0000%	ORF1ab (nsp2)	T85I
SNP	c1912t	N/A	1	100.0000%	ORF1ab (nsp2)	Silent mutation
SNP	c2395t	N/A	1	100.0000%	ORF1ab (nsp2)	Silent mutation
SNP	t2597c	N/A	1	100.0000%	ORF1ab (nsp2)	Silent mutation
SNP	c3037t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c8947t	N/A	1	100.0000%	ORF1ab (nsp4)	Silent mutation
SNP	c12100t	N/A	1	100.0000%	ORF1ab (nsp8)	Silent mutation
SNP	a12878g	N/A	1	100.0000%	ORF1ab (nsp9)	I65V
SNP	c14408t	N/A	1	100.0000%	ORF1ab (nsp12)	P323L
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	t22917g	N/A	1	100.0000%	Spike	L452R
SNP	a23403g	N/A	1	100.0000%	Spike	D614G
SNP	t24349c	N/A	1	100.0000%	Spike	Silent mutation
SNP	g25563t	N/A	1	100.0000%	ORF3a	Q57H
SNP	c26681t	N/A	1	100.0000%	Membrane protein	Silent mutation
SNP	g27459t	N/A	1	100.0000%	ORF7a	E22D
SNP	c27513t	N/A	1	100.0000%	ORF7a	Silent mutation
SNP	c27879t	N/A	1	100.0000%	ORF7b	H42Y
SNP	g27890t	N/A	1	100.0000%	Intergenic (ORF7b/ORF8)	Untranslated
SNP	a28272t	N/A	1	100.0000%	Intergenic (ORF8/ Nucleocapsid)	Untranslated
SNP	g28845a	N/A	1	100.0000%	Nucleocapsid	R191H
SNP	c28887t	N/A	1	100.0000%	Nucleocapsid	T205I

Variant Type	Variant Position and Identified Alternative Base	Coverage¹	Length of Variant	Frequency of Variant¹	Gene (Region)	Amino Acid Mutation
SNP	c29362t	N/A	1	100.0000%	Nucleocapsid	Silent mutation

¹Coverage 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.