

SARS-Related Coronavirus 2, Isolate hCoV-19/Denmark/DCGC-3024/2020

Catalog No. NR-53953

Product Description:

SARS-Related Coronavirus 2, isolate hCoV-19/Denmark/DCGC-3024/2020 was isolated from a human who was exposed to a COVID-19 infected mink in Northern Jutland, Denmark on October 5, 2020. Deposited and labeled as SARS-CoV-2, isolate hCoV-19/hu/DK/CL-5/1 prior to the determination of the official strain name SARS-CoV-2, isolate hCoV-19/Denmark/DCGC-3024/2020. NR-53953 lot 70041232 was produced by infecting *Cercopithecus aethiops* kidney epithelial cells with human signaling lymphocytic activation molecule (Vero-hSLAM) and incubating in Dulbecco's Minimum Essential Medium (ATCC® 30-2002™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) and 1% penicillin/streptomycin solution (ATCC® 30-2300™) for 8 days at 37°C with 5% CO₂. Cell lysate and supernatant was clarified by centrifuging at 1000 × g at 4°C for 10 minutes.

Passage History:

V-hSLAM(2)/V-hSLAM(1) (Statens Serum Institut/BEI Resources); V-hSLAM = Vero cells with human signaling lymphocytic activation molecule

Lot: 70041232

Manufacturing Date: 01JAN2021

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero-hSLAM 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/Denmark/DCGC-3024/2020 (GISAID: EPI_ISL_616802)	99.98% identity with SARS-COV-2, hCoV-19/Denmark/DCGC-3024/2020 (GISAID: EPI_ISL_616802)
Titer by TCID ₅₀ Assay in Vero-hSLAM Cells by Cytopathic Effect ¹ (8 days at 37°C and 5% CO ₂)	Report results	1.6 × 10 ⁴ 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.

/Heather Couch/

Heather Couch

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APPENDIX I: NGS Information for NR-53953 lot 70041232

Sequence analysis using SBC v2.0 pipeline resulted in the discovery of seven SNPs when compared to the reference sequence from EPI_ISL_616802 (see Table I below). Additionally, both the reference sequence EPI_ISL_616802 and NR-53953 lot 70041232 contained nineteen SNPs and four 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-53953 lot 70041232 and reference sequence EPI_ISL_616802

Position in NR-53953_70041232 Sequence	Position in EPI_ISL_616802 Reference Sequence	Position in MN908947 Wuhan-Hu-1 Sequence	Reported MN908947 Wuhan-Hu-1 Sequence	Reported EPI_ISL_616802 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
13362	13362	13422	T	T	C	130	SNP	1	0.070175
15849	15849	15909	T	T	C	68	SNP	1	0.085106
15989	15989	16049	C	C	T	84	SNP	1	0.068966
20173	20173	20233	C	C	A	190	SNP	1	0.175439
22140	22140	22206	A	A	C	288	SNP	1	0.196970
24502	24502	24568	A	A	G	67	SNP	1	0.065574
29299	29299	29365	A	A	T	1031	SNP	1	0.296053

Table II: Variants with different nucleotides between NR-53953 lot 70041232 and GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome)

Position in NR-53953_70041232 Sequence	Position in EPI_ISL_616802 Reference Sequence	Position in MN908947 Wuhan-Hu-1 Sequence	Reported MN908947 Wuhan-Hu-1 Sequence	Reported EPI_ISL_616802 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
187	187	241	C	T	T	N/A	SNP	1	1.000000
462	462	516	TTAT	T	T	N/A	Indel	3	1.000000
2980	2980	3037	C	T	T	N/A	SNP	1	1.000000
5087	5087	5144	C	T	T	N/A	SNP	1	1.000000
6452	6452	6509	AATA	A	A	N/A	Indel	3	1.000000
6478	6478	6538	C	T	T	N/A	SNP	1	1.000000
11716	11716	11776	C	T	T	N/A	SNP	1	1.000000
14348	14348	14408	C	T	T	N/A	SNP	1	1.000000
15596	15596	15656	C	T	T	N/A	SNP	1	1.000000
19895	19895	19955	C	T	T	N/A	SNP	1	1.000000
21705	21705	21765	TACATGT	T	T	N/A	Indel	6	1.000000
22854	22854	22920	A	T	T	N/A	SNP	1	1.000000
23337	23337	23403	A	G	G	N/A	SNP	1	1.000000
23570	23570	23636	A	G	G	N/A	SNP	1	1.000000
24934	24934	25000	C	T	T	N/A	SNP	1	1.000000
25183	25183	25249	G	T	T	N/A	SNP	1	1.000000
25870	25870	25936	C	T	T	N/A	SNP	1	1.000000
25938	25938	26004	C	T	T	N/A	SNP	1	1.000000
28788	28788	28854	C	T	T	N/A	SNP	1	1.000000
28815	28815	28881	G	A	A	N/A	SNP	1	1.000000
28816	28816	28882	G	A	A	N/A	SNP	1	1.000000
22817	22817	28883	G	C	C	N/A	SNP	1	1.000000
29662	29662	29728	TT	T	T	N/A	Indel	1	1.000000