

SARS-Related Coronavirus 2, Isolate hCoV-19/USA/CA_UCSD_5574/2020

Catalog No. NR-54020

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/USA/CA_UCSD_5574/2020 was isolated from a nasopharyngeal swab on December 29, 2020 in San Diego County, California, USA. NR-54020 lot 70042417 was produced by infecting *Homo sapiens* lung adenocarcinoma epithelial cells (Calu-3; ATCC® HTB-55™) and incubating in Eagle's Minimum Essential Medium (ATCC® 30-2003™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) for 4 days at 37°C with 5% CO₂. Cell lysate and supernatant was clarified by centrifuging at 1500 × g for 10 minutes at 4°C.

Passage History:

HBEC(1)/C(1) (University of California San Diego/BEI Resources); HBEC = Human bronchial epithelial cells; C = Calu-3

Lot: 70042417

Manufacturing Date: 08FEB2021

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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_UCSD_5574/2020 (GISAID: EPI_ISL_751801)	99.98% identity with SARS-CoV-2, hCoV-19/USA/CA_UCSD_5574/2020 (GISAID: EPI_ISL_751801)
Titer by TCID₅₀ Assay in Calu-3 Cells by Cytopathic Effect¹ (4 days at 37°C and 5% CO ₂)	Report results	2.8 × 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.

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12 MAY 2021

Program Manager or designee, ATCC Federal Solutions

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APPENDIX I: NGS Information for NR-54020 lot 70042417

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_751801 (see Table I below). Additionally, both the reference sequence EPI_ISL_751801 and NR-54020 lot 70042417 contained thirty-two 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-54020 lot 70042417 and reference sequence EPI_ISL_751801

Position in NR-54020 lot 70042417 Sequence	Position in MN908947 Wuhan-Hu-1 Sequence	Position in EPI_ISL_751801 Reference Sequence	Reported MN908947 Wuhan-Hu-1 Sequence	Reported EPI_ISL_751801 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
1909	1963	1909	T	T	G	59	SNP	1	0.0526
4402	4456	4402	C	C	T	729	SNP	1	0.1938
8767	8821	8767	A	A	G	94	SNP	1	0.05
14616	14679	14616	T	T	C	105	SNP	1	0.0644
15846	15909	15846	T	T	C	125	SNP	1	0.0611

Table II: Variants with different nucleotides between NR-54020 lot 70042417 and GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome)

Position in NR-54020 lot 70042417 Sequence	Position in MN908947 Wuhan-Hu-1 Sequence	Position in EPI_ISL_751801 Reference Sequence	Reported MN908947 Wuhan-Hu-1 Sequence	Reported EPI_ISL_751801 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
187	241	187	C	T	T	5815	SNP	1	1.0000
859	913	859	C	T	T	8073	SNP	1	1.0000
2983	3037	2983	C	T	T	4839	SNP	1	1.0000
3213	3267	3213	C	T	T	5821	SNP	1	1.0000
5334	5388	5334	C	A	A	4646	SNP	1	1.0000
5932	5986	5932	C	T	T	11818	SNP	1	0.997
6900	6954	6900	T	C	C	6472	SNP	1	1.0000
11233	11287	11233	GTCTGGT TTT	G	G	6856.7	Indel	9	1.0000
14345	14408	14345	C	T	T	4270	SNP	1	0.9915
14613	14676	14613	C	T	T	5817	SNP	1	1.0000
15216	15279	15216	C	T	T	7084	SNP	1	1.0000
16113	16176	16113	T	C	C	6863	SNP	1	1.0000
17534	17597	17534	C	T	T	12291	SNP	1	0.9773
17552	17615	17552	A	G	G	12841	SNP	1	1.0000
21702	21765	21702	TACATGT	T	T	4243.3	Indel	6	1.0000
21921	21990	21921	TTTA	T	T	4857.1	Indel	3	1.0000
22991	23063	22991	A	T	T	8926	SNP	1	1.0000
23199	23271	23199	C	A	A	10324	SNP	1	1.0000
23331	23403	23331	A	G	G	10700	SNP	1	1.0000
23532	23604	23532	C	A	A	12883	SNP	1	1.0000
23637	23709	23637	C	T	T	5012	SNP	1	1.0000
24434	24506	24434	T	G	G	5511	SNP	1	1.0000
24842	24914	24842	G	C	C	8872	SNP	1	1.0000
25988	26060	25988	C	T	T	5343	SNP	1	1.0000
26658	26730	26658	G	C	C	8914	SNP	1	0.9886
27900	27972	27900	C	T	T	22961	SNP	1	0.9984

Position in NR-54020 lot 70042417 Sequence	Position in MN908947 Wuhan-Hu-1 Sequence	Position in EPI_ISL_751801 Reference Sequence	Reported MN908947 Wuhan-Hu-1 Sequence	Reported EPI_ISL_751801 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
27976	28048	27976	G	T	T	16233	SNP	1	1.0000
28039	28111	28039	A	G	G	9695	SNP	1	1.0000
28201	28273	28201	AA	A	A	10969	Indel	1	1.0000
28207	28280	28207	G	C	C	9148	SNP	1	1.0000
28208	28281	28208	A	T	T	9234	SNP	1	1.0000
28209	28282	28209	T	A	A	9269	SNP	1	0.9971
28808	28881	28808	G	A	A	12820	SNP	1	1.0000
28809	28882	28809	G	A	A	12205	SNP	1	1.0000
28810	28883	28810	G	C	C	11946	SNP	1	1.0000
28904	28977	28904	C	T	T	14188	SNP	1	0.9975