

SARS-Related Coronavirus 2, Isolate hCoV-19/Singapore/12/2020 (Δ382) (Lineage B)

Catalog No. NR-53885

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/Singapore/12/2020 (Δ382) was collected on February 17, 2020, in Singapore. NR-53885 lot 70041546 was produced by infecting *Cercopithecus aethiops* kidney cells (Vero E6; BEI Resources lot 3956593) with the deposited material and incubating in Dulbecco's Modified Eagle's Medium (ATCC® 30-2002™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) for 5 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:

VE6(3)/VE6(1) (Duke NUS/BEI Resources); VE6 = *Cercopithecus aethiops* kidney cells

Lot: 70041546

Manufacturing Date: 20JAN2021

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TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero E6 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/Singapore/12/2020 (Δ382) (GISAID: EPI_ISL_414378)	99.97% identity with SARS-CoV-2, hCoV-19/Singapore/12/2020 (Δ382) (GISAID: EPI_ISL_414378)
Titer by TCID₅₀ Assay in Vero E6 Cells by Cytopathic Effect¹ (6 days at 37°C and 5% CO ₂)	Report results	2.8 × 10 ⁵ 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.

/Sonia Bjorum Brower/

Sonia Bjorum Brower

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Lead Technical Writer or designee, ATCC Federal Solutions

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APPENDIX I: NGS Information for NR-53885 lot 70041546

Sequence analysis using AMGP readsQC-illumina.py pipeline and freebayes v1.3.1 variant caller resulted in the discovery of six SNPs and one deletion (DEL) when compared to EPI_ISL_414378 (see Table I below). Additionally, NR-53885 lot 70041546 contained two SNPs and one DEL when compared to GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) (see Table II below). There were two reversions observed in which the nucleotide in EPI_ISL_414378 reverted back to the wild-type nucleotide in NR-53885 lot 70041546 (see Table III below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Table I: Variants with different nucleotides between NR-53885 lot 70041546 and EPI_ISL_414378

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	a3625g	2045	1	5.2323%	ORF1ab (nsp3)	Silent mutation
SNP	c13517t	2528	1	15.9810%	ORF1ab (nsp12)	Silent mutation
SNP	t22114c	1245	1	5.2209%	Spike	Silent mutation
SNP	c23525t	2501	1	19.9120%	Spike	H655Y
DEL	Δ23583-23597	3301	-15	38.6852%	Spike	ΔQTQTN (amino acids 675- 679)
SNP	c23606t	3015	1	7.4627%	Spike	R682W
SNP	a29839g	873	1	6.0710%	3'UTR	Untranslated

Table II: Variants with different nucleotides between NR-53885 lot 70041546 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	c8782t	N/A	1	100.0000%	ORF1ab (nsp4)	Silent mutation
SNP	c25568a	N/A	1	100.0000%	ORF3a (nsp2)	A59D
DEL	Δ27848-28229	N/A	-382	100.0000%	ORF7/ORF8	LELQDH [32-37] TFVLF*, Δamino acids 38-44 (ORF7); Δamino acids 1-122(ORF8)

Table III: Variants with different nucleotides between NR-53885 lot 70041546 and EPI_ISL_414378

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	t24011c_rev_t ²	2695	1	98.8868%	Spike	Silent mutation
SNP	c29741t_rev_c	3413	1	97.6560%	3'UTR	Untranslated

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.

²rev = reversion. t24011c represents the original mutation from "t" in Wuhan-Hu-1 to "c" in the provided reference genome. _rev_t represents the reversion back to "t" observed in the sample. This reversion results in the loss of Spike F817L.