

SARS-Related Coronavirus 2, Isolate USA-WA1/2020

Catalog No. NR-52281

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate USA-WA1/2020 was isolated from an oropharyngeal swab from a patient with a respiratory illness who had recently returned from travel to the affected region of China and developed clinical disease (COVID-19) in January 2020 in Washington, USA. NR-52281 lot 70044427 was produced by infecting *Cercopithecus aethiops* kidney cells (Vero E6; ATCC® CRL-1586™) with seed material (lot 70036318) 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(3)/VE6(2) (CDC/BEI Resources); V = Vero cells; VE6 = Vero E6 cells

Lot: 70044427

Manufacturing Date: 16APR2021

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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 isolate USA-WA1/2020 (GenBank: MN985325.1)	99.96% identity with isolate USA-WA1/2020 (GenBank: MN985325.1)
Titer by TCID₅₀ Assay in Vero E6 Cells by Cytopathic Effect¹ (4 days at 37°C and 5% CO ₂)	Report results	1.4 × 10 ⁶ TCID ₅₀ per mL ²
Sterility (21-day incubation) Harpo's HTYE broth, 37°C and 26°C, aerobic ³ 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.

²Titer was determined by cytopathic effects (CPE) and completed in triplicate (1.6 × 10⁶ per mL, 1.6 × 10⁶ per mL and 8.9 × 10⁵ per mL). The average of the three values is reported.

³Atlas, 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-52281 lot 70044427

Sequence analysis using AMGP readsQC-illumina.py pipeline and variant callers LoFreq version: 2.1.5 and freebayes version: v1.3.1-dirty resulted in the discovery of thirteen SNPs when compared to GenBank MN985325.1 (see Table I below). Additionally, both the reference sequence GenBank MN985325.1 and NR-52281 lot 70044427 contained three 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-52281 lot 70044427 and reference sequence GenBank MN985325.1

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	t15357c	3033	1	5.1434%	ORF1ab (nsp12)	Silent mutation
SNP	c17827a	5201	1	19.0540%	ORF1ab (nsp13)	Q531K
SNP	a22110g	1652	1	5.0847%	Spike	Q183R
SNP	a22206g	1859	1	17.8591%	Spike	D215G
SNP	a22296g	2931	1	55.7489%	Spike	H245R
SNP	c22482t	3507	1	20.4733%	Spike	T307I
SNP	c23525t	4368	1	6.2500%	Spike	H655Y
SNP	c23606t	4201	1	19.2811%	Spike	R682G
SNP	g23607t	4189	1	24.8508%	Spike	R682L
SNP	a25806g	1875	1	5.7067%	ORF3a	Silent mutation
SNP	a29839g	1129	1	7.7945%	3'UTR	Untranslated
SNP	a29871c	127	1	22.0472%	3'UTR	Untranslated
SNP	a29871g	127	1	28.3465%	3'UTR	Untranslated

Table II: Variants with different nucleotides between NR-52281 lot 70044427 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	c18060t	N/A	1	100.0000%	ORF1ab (nsp14)	Silent mutation
SNP	t28144c	N/A	1	100.0000%	ORF8	L84S

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.