

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. **Deposited and labeled as 2019 Novel Coronavirus (2019 nCoV) prior to the determination of the official name.** NR-52281 lot 70036318 was produced by infecting *Cercopithecus aethiops* kidney cells (Vero E6; ATCC® CRL-1586™) with the deposited material in Eagle's Minimum Essential Medium (ATCC® 30-2003) supplemented with 2% fetal bovine serum (ATCC® 30-2020) for 6 days at 37°C with 5% CO₂.

Passage History:

V(3)/VE6(1) (Centers for Disease Control and Prevention/BEI Resources); V = Vero cells; VE6 = Vero E6 cells

Lot: 70036318

Manufacturing Date: 28MAY2020

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, isolate USA-WA1/2020 (GenBank: MN985325.1)	99.99% identity with SARS-CoV-2, isolate USA-WA1/2020 (GenBank: MN985325.1)
Sequencing of Species-Specific Region (~ 930 nucleotides)	≥ 98% identity with SARS-CoV-2, isolate USA-WA1/2020 (GenBank: MN985325.1)	100% identity with SARS-CoV-2, isolate USA-WA1/2020 (GenBank: MN985325.1)
(~ 930 nucleotides)	≥ 98% identity with SARS-CoV-2, strain FDAARGOS_983 isolate USA-WA1/2020 (GenBank: MT246667.1)	100% identity with SARS-CoV-2, strain FDAARGOS_983 isolate USA-WA1/2020 (GenBank: MT246667.1)
Titer by TCID₅₀ Assay in Vero E6 Cells by Cytopathic Effect¹ (5 days at 37°C and 5% CO ₂)	Report results	1.6 × 10 ⁶ TCID ₅₀ per mL
Sterility (33-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, 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 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-52281 lot 70036318

Sequence analysis resulted in the discovery of two SNPs when compared to the reference sequence from GenBank MN985325.1. Additionally, both the reference sequence GenBank MN985325.1 and NR-52281_70036318 contained three SNPs when compared to GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) (see Table below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Position in NR-52281_70036318 Sequence	Position in MN985325.1 Reference Sequence	Position in MN908947 Wuhan-Hu-1 Sequence	Reported MN908947 Wuhan-Hu-1 Sequence	Reported MN985325.1 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
8771	8782	8782	C	T	T	n/a	SNP	1	1.0000000
18049	18060	18060	C	T	T	n/a	SNP	1	1.0000000
22471	22482	22482	C	C	T	111	SNP	1	0.1718464
23596	23607	23607	G	G	T	142	SNP	1	0.1593870
28133	28144	28144	T	C	C	n/a	SNP	1	1.0000000