

**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 70034262 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% CO<sub>2</sub>.

**Passage History:**

V(3)/VE6(1) (CDC/BEI Resources); V = Vero cells; VE6 = Vero E6 cells

**Lot: 70034262**

**Manufacturing Date: 23MAR2020**

TEST	SPECIFICATIONS	RESULTS
<b>Identification by Infectivity in Vero E6 Cells</b>	Cell rounding and detachment	Cell rounding and detachment
<b>Next-Generation Sequencing (NGS) of Complete Genome Using Illumina® iSeq™ 100 Platform</b> (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)
<b>Sequencing of Species-Specific Region</b> (~ 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)
<b>Titer by TCID<sub>50</sub> Assay in Vero E6 Cells by Cytopathic Effect<sup>1</sup></b>	Report results	2.8 × 10 <sup>6</sup> TCID <sub>50</sub> per mL in 7 days at 37°C and 5% CO <sub>2</sub>
<b>Sterility (21-day incubation)</b> Harpo's HTYE broth, 37°C and 26°C, aerobic <sup>2</sup> 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
<b>Mycoplasma Contamination</b> 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

<sup>1</sup>The Tissue Culture Infectious Dose 50% (TCID<sub>50</sub>) endpoint is the 50% infectious endpoint in cell culture. The TCID<sub>50</sub> 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% (LD<sub>50</sub>) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID<sub>50</sub> provides a measure of the titer (or infectivity) of a virus preparation.

<sup>2</sup>Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

/Heather Couch/  
Heather Couch

16 SEP 2020

Program Manager or designee, ATCC Federal Solutions

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**APPENDIX I: NGS Information for NR-52281 lot 70034262**

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\_70034262 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_70034262 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