

SARS-Related Coronavirus 2, Isolate USA-CA3/2020

Catalog No. NR-52385

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Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate USA-CA3/2020 was isolated from a nasopharyngeal swab from a human patient on January 29, 2020 in California, USA. NR-52385 lot 70034883 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 4 days at 37°C with 5% CO₂.

Passage History:

V(2)/VE6(2) (CDC/BEI Resources); V = Vero cells; VE6 = Vero E6 cells

Lot: 70034883

Manufacturing Date: 28APR2020

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero E6 Cells	Cell rounding and detachment	Cell rounding and detachment
Sequencing of Species-Specific Region (~ 930 nucleotides)	≥ 98% identity with SARS-CoV-2, isolate USA-CA3/2020 (GenBank: MT027062.1)	100% identity with SARS-CoV-2, isolate USA-CA3/2020 (GenBank: MT027062.1)
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-CA3/2020 (GenBank: MT027062.1)	99.99% identity with SARS-CoV-2, isolate USA-CA3/2020 (GenBank: MT027062.1)
Titer by TCID₅₀ Assay in Vero E6 Cells by Cytopathic Effect¹ (8 days at 37°C and 5% CO ₂)	Report results	1.6 × 10 ⁶ 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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APPENDIX I: NGS Information for NR-52385 lot 70034883

Sequence analysis resulted in the discovery of four SNPs when compared to GenBank MT027062.1 (see Table below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Position in NR-52385_70034883 Sequence	Position in MT027062.1	Reported MT027062.1 Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
11072	11083	G	T	225	SNP	1	1.0000000
21757	21768	A	G	82	SNP	1	0.1943320
21765	21776	G	A	123	SNP	1	0.2064777
21773	21784	T	A	42	SNP	1	0.1752988