

Certificate of Analysis for NR-53515

SARS-Related Coronavirus 2, Isolate New York-PV08449/2020

Catalog No. NR-53515

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate New York-PV08449/2020 was isolated on March 17, 2020 from a nasal swab collected from a patient with a fatal respiratory illness in New York, USA. NR-53515 lot 70036347 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:

VE6(1)/VE6(2) (The Icahn School of Medicine at Mount Sinai Medical School/BEI Resources); VE6 = Vero E6 cells

Lot: 70036347 Manufacturing Date: 23JUN2020

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 NY-PV08449/2020 (GenBank: MT370902.1 and GISAID: EPI_ISL_421400)	100% identity with SARS-CoV-2, isolate NY-PV08449/2020 (GenBank: MT370902.1 and GISAID: EPI_ISL_421400)		
Titer by TCID ₅₀ Assay in Vero E6 Cells by Cytopathic Effect ¹ (5 days at 37°C and 5% CO ₂)	Report results	8.9 × 10 ⁵ TCID ₅₀ per mL		
Sterility (21-day incubation)				
Harpo's HTYE broth, 37°C and 26°C, aerobic ²	No growth	No growth		
Trypticase Soy broth, 37°C and 26°C, aerobic	No growth	No growth		
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth		
Sheep blood agar, 37°C, aerobic	No growth	No growth		
Sheep blood agar, 37°C, anaerobic	No growth	No growth		
Thioglycollate broth, 37°C, anaerobic	No growth	No growth		
DMEM with 10% FBS, 37°C, aerobic	No growth	No growth		
Mycoplasma Contamination				
Agar and broth culture (14-day incubation at 37°C)	None detected	None detected		
DNA detection by PCR of extracted Test Article nucleic acid	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. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

/Heather Couch/ Heather Couch

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Program Manager or designee, ATCC Federal Solutions

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APPENDIX I: NGS Information for NR-53515 lot 70036347

Sequence analysis resulted in the discovery of one SNP when compared to the reference sequence from GISAID EPI_ISL_421400. Additionally, both the reference sequence GISAID EPI_ISL_421400 and NR-53515_70036347 contained seven 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-53515 _70036347 Sequence	Position in EPI_ISL_ 421400 Reference Sequence	Position in MN908947 Wuhan- Hu-1 Sequence	Reported MN908947 Wuhan- Hu-1 Sequence	Reported EPI_ISL_ 421400 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
186	186	241	С	Т	T	n/a	SNP	1	1.0000000
1004	1004	1059	С	Т	Т	n/a	SNP	1	1.0000000
2982	2982	3037	С	Т	Т	n/a	SNP	1	1.0000000
10796	10796	10851	С	Т	Т	n/a	SNP	1	1.0000000
14353	14353	14408	С	Т	Т	n/a	SNP	1	1.0000000
22039	22039	22094	G	G	А	222	SNP	1	0.5
23348	23348	23403	А	G	G	n/a	SNP	1	1.0000000
25508	25508	25563	G	Т	Т	n/a	SNP	1	1.0000000

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