

Certificate of Analysis for NR-52384

SARS-Related Coronavirus 2, Isolate USA-WI1/2020

Catalog No. NR-52384

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate USA-WI1/2020 was isolated from a nasopharyngeal swab from a human patient in Wisconsin, USA on January 31, 2020. NR-52384 lot 70034881 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(3) (Centers for Disease Control and Prevention/BEI Resources); V = Vero cells, VE6 = Vero E6 cells

Lot: 70034881 Manufacturing Date: 18MAY2020

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-WI1/2020 (GenBank: MT039887.1)	99.96% identity with SARS-CoV-2, isolate USA-WI1/2020 (GenBank: MT039887.1)	
Titer by TCID ₅₀ Assay in Vero E6 Cells by Cytopathic Effect ¹ (5 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	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/

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Program Manager or designee, ATCC Federal Solutions

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APPENDIX I: NGS Information for NR-52384 lot 70034881

Sequence analysis resulted in the discovery of two SNPs and a single deletion when compared to GenBank MT039887.1 (see Table below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Position in NR-52384_ 70034881 Sequence	Position in MT039887.1	Reported MT039887.1 Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
11831	11835	С	Т	225	SNP	1	1.0000000
22199	22203	A	G	228	SNP	1	0.9327731
23609	23613	GTAGTGTAGCTAGT	GTAGT	222	Indel	9	0.5271967

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