SUPPORTING INFECTIOUS DISEASE RESEARCH

SARS-Related Coronavirus 2, Isolate USA-IL1/2020

Catalog No. NR-52381

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Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate USA-IL1/2020 was isolated from sputum from a human patient collected on January 21, 2020 in Illinois, USA. NR-52381 lot 70034875 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: 70034875

Manufacturing Date: 04MAY2020

TEST	SPECIFICATIONS	RESULTS		
Identification by Infectivity in Vero E6 Cells	Cell rounding and detachment	Cell rounding and detachment		
Sequencing of Species-Specific Region (~ 870 nucleotides)	≥ 98% identity with SARS-CoV-2, isolate USA-IL1/2020 (GenBank: MN988713.1)	100% identity with SARS-CoV-2, isolate USA-IL1/2020 (GenBank: MN988713.1)		
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-IL1/2020 (GenBank: MN988713.1)	99.92% identity with SARS-CoV- 2, isolate USA-IL1/2020 (GenBank: MN988713.1)		
Titer by TCID ₅₀ Assay in Vero E6 Cells by Cytopathic Effect ¹ (4 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		

¹ The 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. bei resources

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10 AUG 2020

Program Manager or designee, ATCC Federal Solutions

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APPENDIX I: NGS Information for NR-52381 lot 70034875

Sequence analysis resulted in the discovery of ten SNPs and one deletion when compared to GenBank MN988713.1 (see Table below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Position in NR-52381_ 70034875 Sequence	Position in MN988713.1	Reported MN988713.1 Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
486	490	W	A	225	SNP	1	1.0000000
3173	3177	Y	Т	225	SNP	1	1.0000000
8778	8782	Y	Т	225	SNP	1	1.0000000
9514	9518	Т	С	228	SNP	1	0.9957082
22339	22343	G	С	179	SNP	1	0.2622951
23581	23585	CAGACTCAGACTAATT	С	222	Indel	15	0.6134454
24015	24034	Y	Т	225	SNP	1	1.0000000
26710	26729	Y	С	225	SNP	1	1.0000000
28058	28077	S	С	225	SNP	1	1.0000000
28125	28144	Y	С	225	SNP	1	1.0000000
28835	28854	Y	С	225	SNP	1	1.0000000