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SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-52282

SARS-Related Coronavirus 2, Isolate Hong Kong/VM20001061/2020

Catalog No. NR-52282

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate Hong Kong/VM20001061/2020 was isolated from a nasopharyngeal aspirate and throat swab from an adult male patient January 22, 2020 in Hong Kong. NR-52282 lot 70034432 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 5 days at 37°C with 5% CO_2.

Passage History:

VE6(5)/VE6(1) (prior to BEI Resources/BEI Resources); VE6 = Vero E6 cells

Lot: 70034432 **Manufacturing Date: 23MAR2020**

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 hCoV-19/Hong Kong/VM20001061-2/2020 (GISAID: EPI_ISL_412028)	99.9% identity with SARS-CoV-2, isolate hCoV-19/Hong Kong/VM20001061-2/2020 (GISAID: EPI_ISL_412028)
Sequencing of Species-Specific Region (~ 940 nucleotides)	≥ 98% identity with SARS-CoV-2, isolate SARS-CoV-2/human/USA/NR-52282/2020 (GenBank: MT547814.1)	100% identity with SARS-CoV-2, isolate SARS-CoV-2/human/USA/NR-52282/2020 (GenBank: MT547814.1)
Titer by $TCID_{50}$ Assay in Vero E6 Cells by Cytopathic Effect[1] (7 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% ($TCID_{50}$) endpoint is the 50% infectious endpoint in cell culture. The $TCID_{50}$ 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_{50}) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the $TCID_{50}$ provides a measure of the titer (or infectivity) of a virus preparation.

[2]Atlas, Ronald M. Handbook of Microbiological Media. 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-52282 lot 70034432

Sequence analysis resulted in the discovery of seven SNPs and one deletion (indel) when compared to the reference sequence from GISAID EPI_ISL_412028. Additionally, both the reference sequence GISAID EPI_ISL_412028 and NR-52282_70034432 contained six 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-52282_70034432 Sequence	Position in EPI_ISL_412028 Reference Sequence	Position in MN908947 Sequence	Reported MN908947 Sequence	Reported EPI_ISL_412028 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
1650	1650	1663	C	T	T	n/a	SNP	1	1.0000000
8769	8769	8782	C	T	T	n/a	SNP	1	1.0000000
12906	12906	12919	C	C	T	222	SNP	1	0.6201117
21623	21623	21636	C	C	T	219	SNP	1	0.8716578
22648	22648	22661	G	T	T	n/a	SNP	1	1.0000000
23594	23594	23607	G	G	A	228	SNP	1	0.9578947
24021	24021	24034	C	Y	T	225	SNP	1	1.0000000
24553	24553	24566	C	C	G	221	SNP	1	0.7255814
26716	26716	26729	T	C	C	n/a	SNP	1	1.0000000
27250	27250	27263	CTTTTAAA GTTTCCAT TTGGAAT CTTGATT	CTTTTAAA GTTTCCAT TTGGAAT CTTGATT	CTT	221	Indel	27	0.7064220
28037	28064	28077	G	C	C	n/a	SNP	1	1.0000000
28104	28131	28144	T	C	C	n/a	SNP	1	1.0000000
29822	29849	29862	G	T	G	141	SNP	1	1.0000000

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