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SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-54011

SARS-Related Coronavirus 2, Isolate USA/CA_CDC_5574/2020

Catalog No. NR-54011

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate USA/CA_CDC_5574/2020 was isolated from a nasopharyngeal swab on December 29, 2020 in San Diego County, California, USA. NR-54011 lot 70041598 was produced by infecting *Cercopithecus aethiops* kidney epithelial cells (Vero; ATCC® CCL-81™) with the deposited material and incubating 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% CO_2.

Passage History:

V(2)/V(1) (Centers for Disease Control and Prevention/BEI Resources); V = Vero cells

Lot: 70041598 **Manufacturing Date: 19JAN2021**

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero 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, USA/CA_CDC_5574/2020 (GISAID: EPI_ISL_751801)	100% identity with SARS-COV-2, USA/CA_CDC_5574/2020 (GISAID: EPI_ISL_751801)
Titer by $TCID_{50}$ Assay in Vero Cells by Cytopathic Effect[1] (5 days at 37°C and 5% CO_2)	Report results	2.8×10^4 $TCID_{50}$ 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/
Heather Couch 30 MAR 2021
Program Manager or designee, ATCC Federal Solutions

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

Support Provided by NIAID

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APPENDIX I: NGS Information for NR-54011 lot 70041598

Sequence analysis using SBC v2.0 pipeline resulted in the discovery of two SNPs when compared to the reference sequence from EPI_ISL_751801. Additionally, both the reference sequence EPI_ISL_751801 and NR-54011 lot 70041598 contained thirty-two SNPs and four deletions (Indel) 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-54011_ 70041598 Sequence	Position in EPI_ISL_ 751801 Reference Sequence	Position in MN908947 Wuhan-Hu-1 Sequence	Reported MN908947 Wuhan-Hu-1 Sequence	Reported EPI_ISL_ 751801 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
187	187	241	C	T	T	N/A	SNP	1	1.000000
859	859	913	C	T	T	N/A	SNP	1	1.000000
2983	2983	3037	C	T	T	N/A	SNP	1	1.000000
3213	3213	3267	C	T	T	N/A	SNP	1	1.000000
5334	5334	5388	C	A	A	N/A	SNP	1	1.000000
5932	5932	5986	C	T	T	N/A	SNP	1	1.000000
6900	6900	6954	T	C	C	N/A	SNP	1	1.000000
11233	11233	11287	GTCTGGTTTT	G	G	N/A	Indel	9	1.000000
11687	11687	11750	C	C	T	49314	SNP	1	0.996014
14345	14345	14408	C	T	T	N/A	SNP	1	1.000000
14613	14613	14676	C	T	T	N/A	SNP	1	1.000000
14616	14616	14679	T	T	C	1068	SNP	1	0.064356
15216	15216	15279	C	T	T	N/A	SNP	1	1.000000
16113	16113	16176	T	C	C	N/A	SNP	1	1.000000
17534	17534	17597	C	T	T	N/A	SNP	1	1.000000
17552	17552	17615	A	G	G	N/A	SNP	1	1.000000
21702	21702	21765	TACATGT	T	T	N/A	Indel	6	1.000000
21921	21921	21990	TTTA	T	T	N/A	Indel	3	1.000000
22991	22991	23063	A	T	T	N/A	SNP	1	1.000000
23199	23199	23271	C	A	A	N/A	SNP	1	1.000000
23331	23331	23403	A	G	G	N/A	SNP	1	1.000000
23532	23532	23604	C	A	A	N/A	SNP	1	1.000000
23637	23637	23709	C	T	T	N/A	SNP	1	1.000000
24434	24434	24506	T	G	G	N/A	SNP	1	1.000000
24842	24842	24914	G	C	C	N/A	SNP	1	1.000000
25988	25988	26060	C	T	T	N/A	SNP	1	1.000000
26658	26658	26730	G	C	C	N/A	SNP	1	1.000000
27900	27900	27972	C	T	T	N/A	SNP	1	1.000000
27976	27976	28048	G	T	T	N/A	SNP	1	1.000000
28039	28039	28111	A	G	G	N/A	SNP	1	1.000000
28201	28201	28273	AA	A	A	N/A	Indel	1	1.000000
28207	28207	28280	G	C	C	N/A	SNP	1	1.000000
28208	28208	28281	A	T	T	N/A	SNP	1	1.000000
28209	28209	28282	T	A	A	N/A	SNP	1	1.000000
28808	28808	28881	G	A	A	N/A	SNP	1	1.000000
28809	28809	28882	G	A	A	N/A	SNP	1	1.000000
28810	28810	28883	G	C	C	N/A	SNP	1	1.000000
28904	28904	28977	C	T	T	N/A	SNP	1	1.000000

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