Certificate of Analysis for NR-54000

SARS-Related Coronavirus 2, Isolate hCoV-19/England/204820464/2020

Catalog No. NR-54000

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/England/204820464/2020 was isolated from a 58-year-old human male on November 24, 2020 in England, United Kingdom. NR-54000 lot 70041933 was produced by infecting *Cercopithecus aethiops* kidney epithelial cells with human signaling lymphocytic activation molecule (Vero-hSLAM) 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. Cell lysate and supernatant was clarified by centrifuging at 1500 × g for 10 minutes at room temperature.

Passage History:

V-hSLAM(2)/V-hSLAM(1) (Public Health England/BEI Resources); V-hSLAM = Vero-hSLAM

Lot: 70041933 Manufacturing Date: 31JAN2021

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TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Vero-hSLAM 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, hCoV-19/England/204820464/2020 (GISAID: EPI_ISL_683466)	99.95% identity with SARS-COV-2, hCoV-19/England/204820464/2020 (GISAID: EPI_ISL_683466)
Titer by $TCID_{50}$ Assay in Vero-hSLAM Cells by Cytopathic Effect[1] (5 days at 37°C and 5% CO_2)	Report results	8.8 × 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/
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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.

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APPENDIX I: NGS Information for NR-54000 lot 70041933

Sequence analysis using SBC v2.0 pipeline and freebayes v1.3.1 variant caller resulted in the discovery of fifteen SNPs when compared to the reference sequence from EPI_ISL_683466 (see Table I below). Additionally, both the reference sequence EPI_ISL_683466 and NR-54000 lot 70041933 contained thirty-three SNPs and four deletions (Indel) when compared to GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) (see Table II below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

Table I: Variants with different nucleotides between NR-54000 lot 70041933 and reference sequence EPI_ISL_683466

Position in NR-54000 lot 70041933 Sequence	Position in MN908947 Wuhan-Hu-1 Sequence	Position in EPI_ISL_683466 Reference Sequence	Reported MN908947 Wuhan-Hu-1 Sequence	Reported EPI_ISL_683466 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
517	521	518	G	G	T	8621	SNP	1	0.096668
1959	1963	1960	T	T	A	1291	SNP	1	0.057428
1959	1963	1960	T	T	C	1667	SNP	1	0.066417
11401	11414	11402	C	C	T	9979	SNP	1	0.084848
11435	11448	11436	A	A	G	12946	SNP	1	0.097569
11509	11522	11510	T	T	G	9979	SNP	1	0.084848
11860	11873	11861	G	G	A	26843	SNP	1	0.235626
13326	13339	13327	T	T	G	11434	SNP	1	0.079975
14666	14679	14667	T	T	C	1529	SNP	1	0.061818
17873	17886	17874	T	T	C	2714	SNP	1	0.062846
21666	21679	21667	T	T	C	3263	SNP	1	0.050658
22092	22114	22093	T	T	C	2402	SNP	1	0.086573
22968	22990	22969	T	T	C	9221	SNP	1	0.076779
25784	25806	25785	A	A	G	1932	SNP	1	0.052653
29817	29839	29818	A	A	G	872	SNP	1	0.056749

Table II: Variants with different nucleotides between NR-54000 lot 70041933 and GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome)

Position in NR-54000 lot 70041933 Sequence	Position in MN908947 Wuhan-Hu-1 Sequence	Position in EPI_ISL_683466 Reference Sequence	Reported MN908947 Wuhan-Hu-1 Sequence	Reported EPI_ISL_683466 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
237	241	238	C	T	T	N/A	SNP	1	1.00000
909	913	910	C	T	T	N/A	SNP	1	1.00000
3033	3037	3034	C	T	T	N/A	SNP	1	1.00000
3263	3267	3264	C	T	T	N/A	SNP	1	1.00000
4995	4999	4996	C	Y	T	N/A	SNP	1	1.00000
5384	5388	5385	C	A	A	N/A	SNP	1	1.00000
5982	5986	5983	C	T	T	N/A	SNP	1	1.00000
6629	6633	6630	C	T	T	N/A	SNP	1	1.00000
6950	6954	6951	T	C	C	N/A	SNP	1	1.00000
11283	11287	11282	GTCTGGTTTT	G	G	142130	Indel	9	1.00000
14395	14408	14396	C	T	T	N/A	SNP	1	1.00000
14663	14676	14664	C	T	T	N/A	SNP	1	1.00000
15266	15279	15267	C	T	T	N/A	SNP	1	1.00000
16163	16176	16164	T	C	C	N/A	SNP	1	1.00000
17602	17615	17603	A	G	G	N/A	SNP	1	1.00000
19066	19079	19067	A	G	G	N/A	SNP	1	1.00000

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Position in NR-54000 lot 70041933 Sequence	Position in MN908947 Wuhan-Hu-1 Sequence	Position in EPI_ISL_683466 Reference Sequence	Reported MN908947 Wuhan-Hu-1 Sequence	Reported EPI_ISL_683466 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
19841	19854	19842	C	T	T	N/A	SNP	1	1.00000
21752	21765	21753	TACATGT	T	T	123785	Indel	7	1.00000
21969	21990	21972	TTTA	T	T	121724	Indel	3	1.00000
23041	23063	23042	A	T	T	N/A	SNP	1	1.00000
23249	23271	23250	C	A	A	N/A	SNP	1	1.00000
23381	23403	23382	A	G	G	N/A	SNP	1	1.00000
23582	23604	23583	C	A	A	N/A	SNP	1	1.00000
23687	23709	23688	C	T	T	N/A	SNP	1	1.00000
24484	24506	24485	T	G	G	N/A	SNP	1	1.00000
24892	24914	24893	G	C	C	N/A	SNP	1	1.00000
27950	27972	27951	C	T	T	N/A	SNP	1	1.00000
28026	28048	28027	G	T	T	N/A	SNP	1	1.00000
28089	28111	28090	A	G	G	N/A	SNP	1	1.00000
28249	28270	28249	TA	T	T	219	Indel	1	1.00000
28258	28280	28259	G	C	C	N/A	SNP	1	1.00000
28259	28281	28260	A	T	T	N/A	SNP	1	1.00000
28260	28282	28261	T	A	A	N/A	SNP	1	1.00000
28859	28881	28860	G	A	A	N/A	SNP	1	1.00000
28860	28882	28861	G	A	A	N/A	SNP	1	1.00000
28861	28883	28862	G	C	C	N/A	SNP	1	1.00000
28955	28977	28956	C	T	T	N/A	SNP	1	1.00000

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