

SARS-Related Coronavirus 2, Isolate hCoV-19/South Africa/KRISP-K005325/2020

Catalog No. NR-54009

Product Description:

NR-54009 lot 70041942 was produced by infecting *Cercopithecus aethiops* kidney epithelial cells with human signaling lymphocytic activation molecule (Vero-hSLAM) with severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/South Africa/KRISP-K005325/2020 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% CO₂. Cell lysate and supernatant was clarified by centrifuging at 1500 × g for 10 minutes at room temperature.

Passage History:

H(1)VE(2)/V-hSLAM(1) (African Health Research Institute/BEI Resources); H = H1299 cells overexpressing human ACE2 receptor (H1299-hACE2-E3); VE = Vero E6 cells; V-hSLAM = Vero-hSLAM cells

Lot: 70041942

Manufacturing Date: 31JAN2021

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/South Africa/KRISP-K005325/2020 (GISAID: EPI_ISL_678615)	99.96% identity with SARS-COV-2, hCoV-19/South Africa/KRISP-K005325/2020 (GISAID: EPI_ISL_678615)
Titer by TCID₅₀ Assay in Vero-hSLAM Cells by Cytopathic Effect¹ (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 Trypticase Soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C, aerobic	No growth No growth No growth No growth No growth No growth No growth	No growth No growth No growth No growth No growth No growth No growth
Mycoplasma Contamination Agar and broth culture (14-day incubation at 37°C) DNA detection by PCR of extracted Test Article nucleic acid	None detected None detected	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. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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30 MAR 2021

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APPENDIX I: NGS Information for NR-54009 lot 70041942

Sequence analysis using SBC v2.0 pipeline and freebayes v1.3.1 variant caller resulted in the discovery of twenty SNPs when compared to the reference sequence from EPI_ISL_678615 (see Table I below). Additionally, both the reference sequence EPI_ISL_678615 and NR-54009 lot 70041942 contained twenty-three SNPs and two 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-54009 lot 70041942 and reference sequence EPI_ISL_678615

Position in NR-54009_70041942 Sequence	Position in EPI_ISL_678615 Reference Sequence	Position in MN908947 Wuhan-Hu-1 Sequence	Reported MN908947 Wuhan-Hu-1 Sequence	Reported EPI_ISL_678615 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
1954	1961	1963	T	T	A	910	SNP	1	0.058978
10800	10807	10809	C	C	T	33392	SNP	1	0.543260
11011	11018	11020	C	C	T	16030	SNP	1	0.247118
11732	11739	11750	C	C	T	12255	SNP	1	0.100692
13321	13328	13339	T	T	G	28841	SNP	1	0.255795
14661	14668	14679	T	T	C	5481	SNP	1	0.184303
17321	17328	17339	C	C	T	11935	SNP	1	0.071670
21633	21640	21651	A	A	C	15458	SNP	1	0.115539
22096	22103	22114	T	T	C	2159	SNP	1	0.108139
23566	23573	23593	G	G	T	49314	SNP	1	0.898592
23579	23586	23606	C	C	T	49314	SNP	1	0.902460
25779	25786	25806	A	A	G	1079	SNP	1	0.051010
25783	25790	25810	C	C	T	6728	SNP	1	0.140390
26795	26802	26822	C	C	T	7617	SNP	1	0.069952
26867	26874	26894	C	C	T	8233	SNP	1	0.058733
27366	27373	27393	C	C	T	49314	SNP	1	0.631057
27600	27607	27627	T	T	A	49068	SNP	1	0.283398
28210	28217	28237	G	G	T	49314	SNP	1	0.900036
28341	28348	28368	G	G	A	14856	SNP	1	0.090313
29796	29801	29821	T	T	G	14220	SNP	1	0.120315

Table II: Variants with different nucleotides between NR-54009 lot 70041942 and GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome)

Position in NR-54009_70041942 Sequence	Position in EPI_ISL_678615 Reference Sequence	Position in MN908947 Wuhan-Hu-1 Sequence	Reported MN908947 Wuhan-Hu-1 Sequence	Reported EPI_ISL_678615 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
165	172	174	G	T	T	N/A	SNP	1	1.000000
232	239	241	C	T	T	N/A	SNP	1	1.000000
1050	1057	1059	C	T	T	N/A	SNP	1	1.000000
2683	2690	2692	A	T	T	N/A	SNP	1	1.000000
3028	3035	3037	C	T	T	N/A	SNP	1	1.000000
5221	5228	5230	G	T	T	N/A	SNP	1	1.000000
10314	10321	10323	A	G	G	N/A	SNP	1	1.000000
11278	11285	11287	GTCTGGT TTT	G	G	77148.6	Indel	9	1.000000
14390	14397	14408	C	T	T	N/A	SNP	1	1.000000
21596	21603	21614	C	T	T	N/A	SNP	1	1.000000
21783	21790	21801	A	C	C	N/A	SNP	1	1.000000

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Position in NR-54009_70041942 Sequence	Position in EPI_ISL_678615 Reference Sequence	Position in MN908947 Wuhan-Hu-1 Sequence	Reported MN908947 Wuhan-Hu-1 Sequence	Reported EPI_ISL_678615 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
22188	22195	22206	A	G	G	N/A	SNP	1	1.000000
22263	22270	22290	TTTACTTG C	C	C	111362	Indel	9	1.000000
22786	22793	22813	G	T	T	N/A	SNP	1	1.000000
22985	22992	23012	G	A	A	N/A	SNP	1	1.000000
23036	23043	23063	A	T	T	N/A	SNP	1	1.000000
23376	23383	23403	A	G	G	N/A	SNP	1	1.000000
23637	23644	23664	C	T	T	N/A	SNP	1	1.000000
25536	25543	25563	G	T	T	N/A	SNP	1	1.000000
25757	25764	25784	G	T	T	N/A	SNP	1	1.000000
25877	25884	25904	C	T	T	N/A	SNP	1	1.000000
26429	26436	26456	C	T	T	N/A	SNP	1	1.000000
27643	27650	27670	G	T	T	N/A	SNP	1	1.000000
28226	28233	28253	C	T	T	N/A	SNP	1	1.000000
28860	28867	28887	C	T	T	N/A	SNP	1	1.000000