

**SARS-Related Coronavirus 2, Isolate hCoV-19/South Africa/KRISP-EC-K005321/2020**

**Catalog No. NR-54008**

**Product Description:**

NR-54008 lot 70041939 was produced by infecting *Cercopithecus aethiops* kidney epithelial cells with human signaling lymphocytic activation molecule (Vero-hSLAM) with SARS-Related Coronavirus 2, Isolate hCoV-19/South Africa/KRISP-EC-K005321/2020 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% CO<sub>2</sub>. 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-hACE2-E3 cells; VE = Vero E6 cells; V-hSLAM = Vero-hSLAM cells

**Lot: 70041939**

**Manufacturing Date: 31JAN2021**

TEST	SPECIFICATIONS	RESULTS
<b>Identification by Infectivity in Vero-hSLAM Cells</b>	Cell rounding and detachment	Cell rounding and detachment
<b>Next-Generation Sequencing (NGS) of Complete Genome Using Illumina® iSeq™ 100 Platform</b> (Refer to Appendix I for NGS information)	≥ 98% identity with SARS-COV-2, hCoV-19/South Africa/KRISP-EC-K005321/2020 (GISAID: EPI_ISL_678570)	99.97% identity with SARS-COV-2, hCoV-19/South Africa/KRISP-EC-K005321/2020 (GISAID: EPI_ISL_678570)
<b>Titer by TCID<sub>50</sub> Assay in Vero-hSLAM Cells by Cytopathic Effect<sup>1</sup></b> (5 days at 37°C and 5% CO <sub>2</sub> )	Report results	2.8 × 10 <sup>6</sup> TCID <sub>50</sub> per mL
<b>Sterility (21-day incubation)</b> Harpo's HTYE broth, 37°C and 26°C, aerobic <sup>2</sup> 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
<b>Mycoplasma Contamination</b> 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

<sup>1</sup>The Tissue Culture Infectious Dose 50% (TCID<sub>50</sub>) endpoint is the 50% infectious endpoint in cell culture. The TCID<sub>50</sub> 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<sub>50</sub>) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the TCID<sub>50</sub> provides a measure of the titer (or infectivity) of a virus preparation.

<sup>2</sup>Atlas, 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-54008 lot 70041939**

Sequence analysis using SBC v2.0 pipeline and freebayes v1.3.1 variant caller resulted in the discovery of seven SNPs and one deletion (Indel) when compared to the reference sequence from EPI\_ISL\_678570 (see Table I below). Additionally, both the reference sequence EPI\_ISL\_678570 and NR-54008 lot 70041939 contained twenty SNPs and two deletions 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-54008 lot 70041939 and reference sequence EPI\_ISL\_678570**

Position in NR-54008 lot 70041939 Sequence	Position in EPI_ISL_678570 Reference Sequence	Position in MN908947 Wuhan-Hu-1 Sequence	Reported MN908947 Wuhan-Hu-1 Sequence	Reported EPI_ISL_678570 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
2054	2059	2062	C	C	T	222	SNP	1	0.056047
10370	10375	10378	A	A	C	913	SNP	1	0.086806
11733	11738	11750	C	C	T	665	SNP	1	0.068017
11881	11886	11898	A	A	G	8770	SNP	1	0.854286
21742	21747	21759	A	A	G	6005	SNP	1	0.477895
22097	22102	22114	T	T	C	176	SNP	1	0.056716
23562	23567	23588	ACTCAGA CTAATTCT CC	ACTCAGA CTAATTCT CC	AC	23958.2	Indel	15	1.00000
27663	27668	27689	C	C	A	285	SNP	1	0.88889

**Table II: Variants with different nucleotides between NR-54008 lot 70041939 and GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome)**

Position in NR-54008 lot 70041939 Sequence	Position in EPI_ISL_678570 Reference Sequence	Position in MN908947 Wuhan-Hu-1 Sequence	Reported MN908947 Wuhan-Hu-1 Sequence	Reported EPI_ISL_678570 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
166	171	174	G	T	T	N/A	SNP	1	1.00000
233	238	241	C	T	T	N/A	SNP	1	1.00000
1051	1056	1059	C	T	T	N/A	SNP	1	1.00000
3029	3034	3037	C	T	T	N/A	SNP	1	1.00000
5222	5227	5230	G	T	T	N/A	SNP	1	1.00000
10315	10320	10323	A	G	G	N/A	SNP	1	1.00000
11278	11284	11287	GTCTGGT TTT	G	G	11174.1	Indel	9	1.00000
14391	14396	14408	C	T	T	N/A	SNP	1	1.00000
21784	21789	21801	A	C	C	N/A	SNP	1	1.00000
22189	22194	22206	A	G	G	N/A	SNP	1	1.00000
22264	22269	22290	TTTACTTG C	C	C	19451.1	Indel	9	1.00000
22787	22792	22813	G	T	T	N/A	SNP	1	1.00000
22986	22991	23012	G	A	A	N/A	SNP	1	1.00000
23037	23042	23063	A	T	T	N/A	SNP	1	1.00000
23377	23382	23403	A	G	G	N/A	SNP	1	1.00000
23638	23643	23664	C	T	T	N/A	SNP	1	1.00000
25537	25542	25563	G	T	T	N/A	SNP	1	1.00000
25878	25883	25904	C	T	T	N/A	SNP	1	1.00000
26430	26435	26456	C	T	T	N/A	SNP	1	1.00000
27644	27649	27670	G	N	T	N/A	SNP	1	1.00000

## Certificate of Analysis for NR-54008

Position in NR-54008 lot 70041939 Sequence	Position in EPI_ISL_678570 Reference Sequence	Position in MN908947 Wuhan-Hu-1 Sequence	Reported MN908947 Wuhan-Hu-1 Sequence	Reported EPI_ISL_678570 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
28227	28233	28253	C	T	T	N/A	SNP	1	1.00000
28861	28867	28887	C	T	T	N/A	SNP	1	1.00000