

Certificate of Analysis for NR-53945

SARS-Related Coronavirus 2, Isolate hCoV-19/Scotland/CVR2224/2020

Catalog No. NR-53945

Product Description:

SARS-Related Coronavirus 2, isolate hCoV-19/Scotland/CVR2224/2020 was isolated from a throat swab from a human patient diagnosed with COVID-19 on July 17, 2020 in Scotland, United Kingdom. Deposited and labeled as SARS-CoV-2, isolate CVR-GLA-2 prior to the determination of the official strain name SARS-CoV-2, isolate hCoV-19/Scotland/CVR2224/2020. NR-53945 lot 70041279 was produced by infecting *Cercopithecus aethiops* kidney epithelial cells with human signaling lymphocytic activation molecule (Vero-hSLAM) and incubating in Dulbecco's Minimum Essential Medium (ATCC® 30-2003™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) and 1% penicillin/streptomycin (ATCC® 30-2300™) solution for 5 days at 37°C with 5% CO₂.

Passage History:

VE(1)/VhSLAM(1) (University of Glasgow Centre for Virus Research/BEI Resources); VE = Vero E6 cells; VhSLAM = Vero-hSLAM cells

Lot: 70041279 Manufacturing Date: 29DEC2020

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TEST	SPECIFICATIONS	RESULTS Cell rounding and detachment 99.95% identity with SARS- CoV-2, isolate hCoV- 19/Scotland/CVR2224/2020 (GISAID: EPI_ISL_448167)		
Identification by Infectivity in Vero-hSLAM Cells	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/Scotland/CVR2224/2020 (GISAID: EPI_ISL_448167)			
Titer by TCID₅ Assay in Vero-hSLAM Cells by Cytopathic Effect¹ (7 days at 37°C and 5% CO₂)	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		

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. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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27 APR 2021

Program Manager or designee, ATCC Federal Solutions

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APPENDIX I: NGS Information for NR-53945 lot 70041279

Sequence analysis using SBC v2.0 pipeline resulted in the discovery of fourteen SNPs when compared to the reference sequence from EPI_ISL_448167 (see Table I below). Additionally, both the reference sequence EPI_ISL_448167 and NR-53945 lot 70041279 contained six SNPs 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-53945 lot 70041279 and reference sequence EPI ISL 448167

Position in NR-53945_ 70041279 Sequence	Position in MN908947 Wuhan- Hu-1 Sequence	Position in EPI_ISL_ 448167 Reference Sequence	Reported MN908947 Wuhan- Hu-1 Sequence	Reported EPI_ISL_ 448167 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
1909	1963	1909	Т	Т	G	294	SNP	1	0.062350
2443	2497	2443	Α	Α	G	741	SNP	1	0.065705
3028	3082	3028	Α	Α	G	452	SNP	1	0.070081
9306	9360	9306	С	С	Т	301	SNP	1	0.071111
12693	12747	12693	С	С	Т	1006	SNP	1	0.053663
14237	14291	14237	Α	Α	С	1321	SNP	1	0.062185
14625	14679	14625	Т	Т	С	709	SNP	1	0.145038
16335	16389	16335	Т	Т	Α	3176	SNP	1	0.231465
19670	19724	19670	Т	Т	С	20857	SNP	1	1.000000
22151	22205	22151	G	G	С	1443	SNP	1	0.158621
22152	22206	22152	Α	Α	G	790	SNP	1	0.100457
23471	23525	23471	С	С	Т	3694	SNP	1	0.123043
23553	23607	23553	G	G	Т	1142	SNP	1	0.060489
26150	26204	26150	С	С	Т	1055	SNP	1	0.081818

Table II: Variants with different nucleotides between NR-53945 lot 70041279 and GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome)

Position in NR-53945_ 70041279 Sequence	Position in MN908947 Wuhan- Hu-1 Seguence	Position in EPI_ISL_ 448167 Reference Sequence	Reported MN908947 Wuhan- Hu-1 Sequence	Reported EPI_ISL_ 448167 Reference Seguence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
187	241	187	С	T	T	N/A	SNP	1	1.000000
2983	3037	2983	С	Т	Т	N/A	SNP	1	1.000000
14354	14408	14354	С	Т	Т	N/A	SNP	1	1.000000
22825	22879	22825	С	Α	А	N/A	SNP	1	1.000000
23349	23403	23349	Α	G	G	N/A	SNP	1	1.000000
29519	29573	29519	G	Т	Т	N/A	SNP	1	1.000000

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