

SARS-Related Coronavirus 2, Isolate hCoV-19/Peru/un-CDC-2-4069945/2021 (Lineage C.37; Lambda Variant)

Catalog No. NR-55654

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/Peru/un-CDC-2-4069945/2021 was isolated in Peru on February 20, 2021. NR-55654 lot 70045858 was produced by infecting *Homo sapiens* lung adenocarcinoma epithelial cells (Calu-3; ATCC® HTB-55™) 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 3 days at 37°C with 5% CO₂. The cells and supernatant were spin-clarified at 1500 × g for 10 minutes at 4°C.

Passage History:

VT(1)/C(1) (Centers for Disease Control and Prevention/BEI Resources); VT = *Cercopithecus aethiops* kidney cells with transmembrane protease, serine 2 gene (TMPRSS2); C = Calu-3

Lot: 70045858

Manufacturing Date: 28JUN2021

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TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in Calu-3 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/Peru/un-CDC-2-4069945/2021 (GISAID: EPI_ISL_1591097)	100% identity with SARS-CoV-2, hCoV-19/Peru/un-CDC-2-4069945/2021 (GISAID: EPI_ISL_1591097)
Titer by TCID₅₀ Assay in Calu-3 Cells by Cytopathic Effect¹ (6 days at 37°C and 5% CO ₂)	Report results	2.0 × 10 ⁶ TCID ₅₀ per mL ²
Endotoxin Content (<i>Limulus</i> Amoebocyte Lysate Assay)	Report results	≤ 0.03 EU per mL
Sterility (21-day incubation) Harpo's HTYE broth, 37°C and 26°C, aerobic ³ 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.

²Titer was determined by cytopathic effects (CPE) and completed in triplicate (1.6 × 10⁶ per mL, 1.6 × 10⁶ per mL and 2.8 × 10⁶ per mL). The average of the three values is reported.

³Atlas, Ronald M. *Handbook of Microbiological Media*. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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APPENDIX I: NGS Information for NR-55654 lot 70045858

Sequence analysis using SBC v2.0 pipeline and freebayes v1.3.1 variant caller resulted in the discovery of twenty-eight SNPs and two deletions (DEL) in both the reference sequence EPI_ISL_1591097 and NR-55654 lot 70045858 when compared to GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) (see Table I below).

Table I: Variants with different nucleotides between NR-55654 lot 70045858 and GenBank MN908947.3 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome)

Variant Type	Variant Position and Identified Alternative Base	Coverage ¹	Length of Variant	Frequency of Variant ¹	Gene (Region)	Amino Acid Mutation
SNP	c241t	N/A	1	100.0000%	5'UTR	Untranslated
SNP	c3037t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c4002t	N/A	1	100.0000%	ORF1ab (nsp3)	T428I
SNP	c7124t	N/A	1	100.0000%	ORF1ab (nsp3)	P1469S
SNP	t7424g	N/A	1	100.0000%	ORF1ab (nsp3)	F1569V
SNP	c9857t	N/A	1	100.0000%	ORF1ab (nsp4)	Silent mutation
SNP	t9867c	N/A	1	100.0000%	ORF1ab (nsp4)	L438P
SNP	c10029t	N/A	1	100.0000%	ORF1ab (nsp4)	T492I
SNP	g10097a	N/A	1	100.0000%	ORF1ab (nsp5)	G15S
DEL	Δ11288-11296	N/A	-9	100.0000%	ORF1ab (nsp6)	ΔSGF (amino acids 106-108)
SNP	c13536t	N/A	1	100.0000%	ORF1ab (nsp12)	Silent mutation
SNP	c14408t	N/A	1	100.0000%	ORF1ab (nsp12)	P323L
SNP	g21786t	N/A	1	100.0000%	Spike	G75V
SNP	c21789t	N/A	1	100.0000%	Spike	T76I
DEL	Δ22299-22319	N/A	-21	100.0000%	Spike	R246N, ΔSYLTPGD (amino acids 247-253) ²
SNP	t22917a	N/A	1	100.0000%	Spike	L452Q
SNP	t23031c	N/A	1	100.0000%	Spike	F490S
SNP	a23403g	N/A	1	100.0000%	Spike	D614G
SNP	c23731t	N/A	1	100.0000%	Spike	Silent mutation
SNP	c24138a	N/A	1	100.0000%	Spike	T859N
SNP	c26894t	N/A	1	100.0000%	Membrane protein	Silent mutation
SNP	c28253t	N/A	1	100.0000%	ORF8	Silent mutation
SNP	a28271t	N/A	1	100.0000%	Intergenic (ORF8/N)	Untranslated

Variant Type	Variant Position and Identified Alternative Base	Coverage ¹	Length of Variant	Frequency of Variant ¹	Gene (Region)	Amino Acid Mutation
SNP	c28311t	N/A	1	100.0000%	Nucleocapsid	P13L
SNP	g28881a	N/A	1	100.0000%	Nucleocapsid	R203K
SNP	g28882a	N/A	1	100.0000%		
SNP	g28883c	N/A	1	100.0000%	Nucleocapsid	G204R
SNP	g28913t	N/A	1	100.0000%	Nucleocapsid	G214C
SNP	c29311t	N/A	1	100.0000%	Nucleocapsid	Silent mutation
SNP	c29370t	N/A	1	100.0000%	Nucleocapsid	T366I

¹Coverage for all variants in Table I is listed as 'N/A'. There is no read coverage information for these variants because the sample reads are only mapped to the reference sequence and not to the SARS-CoV-2, Wuhan-Hu-1 isolate sequence (GenBank MN908947), but that does not mean these areas lack for coverage. All variants in Table I are mismatches in between the reference sequence and the SARS-CoV-2, Wuhan-Hu-1 sequence, so these variants will be assigned a frequency of 100% and will therefore be majority SNPs.

²This deletion begins with the third base pair of codon 247 and ends at the second base pair of codon 253, resulting in the deletion of amino acids 247 to 253. The remaining 3 base pairs create of a new amino acid in place of the original amino acids.