

SARS-Related Coronavirus 2, Isolate hCoV-19/USA/OR-OHSU-PHL00037/2021 (Lineage B.1.1.7; Alpha Variant)

Catalog No. NR-55461

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/USA/OR-OHSU-PHL00037/2021 was isolated from a nasopharyngeal swab on February 6, 2021 in Marion County, Oregon, USA. NR-55461 lot 70044271 was produced by infecting *Homo sapiens* lung adenocarcinoma epithelial cells (Calu-3; ATCC® HTB-55™) 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 4°C.

Passage History:

VT(1)/C(1) (Centers for Disease Control and Prevention/BEI Resources); VT = *Cercopithecus aethiops* Kidney Epithelial Cells with Transmembrane Protease, Serine 2 Gene; C = Calu-3

Lot: 70044271

Manufacturing Date: 27APR2021

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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/USA/OR-OHSU-PHL00037/2021 (GISAID: EPI_ISL_1055380)	99.93% identity with SARS-COV-2, hCoV-19/USA/OR-OHSU-PHL00037/2021 (GISAID: EPI_ISL_1055380)
Titer by TCID₅₀ Assay in Calu-3 Cells by Cytopathic Effect¹ (5 days at 37°C and 5% CO ₂)	Report results	1.6 × 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 1.6 × 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-55461 lot 70044271

Sequence analysis using SBC v2.0 pipeline and freebayes v1.3.1 variant caller resulted in the discovery of twenty-two SNPs when compared to the reference sequence from EPI_ISL_1055380 (see Table I below). Additionally, both the reference sequence EPI_ISL_1055380 and NR-55462 lot 70044274 contained thirty-nine SNPs and four deletions (DEL) when compared to GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) (see Table II below).

Table I: Variants with different nucleotides between NR-55461 lot 70044271 and reference sequence EPI_ISL_1055380

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	t306c	136	1	8.0882%	ORF1ab (nsp1)	V14A
SNP	a1350g	393	1	5.5980%	ORF1ab (nsp2)	Q182R
SNP	t1963c	191	1	9.4241%	ORF1ab (nsp2)	Silent mutation
SNP	t1963g	191	1	7.3298%	ORF1ab (nsp2)	Silent mutation
SNP	t3721c	141	1	7.0922%	ORF1ab (nsp3)	Silent mutation
SNP	g7393a	534	1	5.2434%	ORF1ab (nsp3)	Silent mutation
SNP	t8323c	184	1	7.6087%	ORF1ab (nsp3)	Silent mutation
SNP	t10082c	298	1	6.7114%	ORF1ab (nsp5)	S10P
SNP	a10451g	390	1	7.1795%	ORF1ab (nsp5)	N133D
SNP	c12747t	598	1	53.6789%	ORF1ab (nsp9)	T21I
SNP	t13422c	841	1	7.3722%	ORF1ab (nsp10)	L133P
SNP	g13427c	374	1	8.0214%	ORF1ab (nsp10)	E135Q
SNP	c13436t	78	1	5.1282%	ORF1ab (nsp10)	L138F
SNP	a13949g	156	1	5.1282%	ORF1ab (nsp12)	D170G
SNP	t14679c	166	1	6.0241%	ORF1ab (nsp12)	Silent mutation
SNP	t19896c	768	1	10.2865%	ORF1ab (nsp15)	Silent mutation
SNP	t22990c	442	1	13.5747%	Spike	Silent mutation
SNP	t23986c	330	1	8.1818%	Spike	Silent mutation
SNP	t25119c	216	1	6.0185%	Spike	L1186P
SNP	a25806g	244	1	6.5574%	ORF3a	Silent mutation
SNP	t28061a	369	1	6.5041%	ORF8	Silent mutation
SNP	t28061g	369	1	5.6911%	ORF8	Silent mutation

Table II: Variants with different nucleotides between NR-55461 lot 70044271 and GenBank MN908947 (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 region
SNP	c913t	N/A	1	100.0000%	ORF1ab (nsp2)	Silent mutation

Variant Type	Variant Position and Identified Alternative Base	Coverage ¹	Length of Variant	Frequency of Variant ¹	Gene (Region)	Amino Acid Mutation
SNP	g1772a	N/A	1	100.0000%	ORF1ab (nsp2)	V323I
SNP	c2110t	N/A	1	100.0000%	ORF1ab (nsp2)	Silent mutation
SNP	c3037t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c3267t	N/A	1	100.0000%	ORF1ab (nsp3)	T183I
SNP	t5077c	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	c5388a	N/A	1	100.0000%	ORF1ab (nsp3)	A890D
SNP	c5986t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	t6954c	N/A	1	100.0000%	ORF1ab (nsp3)	I1412T
SNP	c7858t	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	t7984c	N/A	1	100.0000%	ORF1ab (nsp3)	Silent mutation
DEL	Δ11288-11296	N/A	-9	100.0000%	ORF1ab (nsp6)	ΔSGF (amino acids 106-108)
SNP	t12226c	N/A	1	100.0000%	ORF1ab (nsp8)	Silent mutation
SNP	c14120t	N/A	1	100.0000%	ORF1ab (nsp12)	P227L
SNP	c14408t	N/A	1	100.0000%	ORF1ab (nsp12)	P323L
SNP	c14676t	N/A	1	100.0000%	ORF1ab (nsp12)	Silent mutation
SNP	c15279t	N/A	1	100.0000%	ORF1ab (nsp12)	Silent mutation
SNP	t16176c	N/A	1	100.0000%	ORF1ab (nsp12)	Silent mutation
SNP	c19390t	N/A	1	100.0000%	ORF1ab (nsp14)	P451S
DEL	Δ21765-21770	N/A	-6	100.0000%	Spike	ΔHV (amino acids 69-70)
DEL	Δ21991-21993	N/A	-3	100.0000%	Spike	ΔY (amino acid 144)
SNP	g23012a	N/A	1	100.0000%	Spike	E484K
SNP	a23063t	N/A	1	100.0000%	Spike	N501Y
SNP	c23271a	N/A	1	100.0000%	Spike	A570D
SNP	a23403g	N/A	1	100.0000%	Spike	D614G
SNP	c23604a	N/A	1	100.0000%	Spike	P681H
SNP	c23709t	N/A	1	100.0000%	Spike	T716I
SNP	t24506g	N/A	1	100.0000%	Spike	S982A
SNP	g24914c	N/A	1	100.0000%	Spike	D1118H
SNP	c27972t	N/A	1	100.0000%	ORF8	Q27*
SNP	g28048t	N/A	1	100.0000%	ORF8	R52I
SNP	a28095t	N/A	1	100.0000%	ORF8	K68*
SNP	a28111g	N/A	1	100.0000%	ORF8	Y73C
DEL	Δ28271	N/A	-1	100.0000%	Intergenic (ORF8/ Nucleocapsid)	Untranslated region
SNP	g28280c	N/A	1	100.0000%	Nucleocapsid	D3L
SNP	a28281t	N/A	1	100.0000%		
SNP	t28282a	N/A	1	100.0000%		
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	c28977t	N/A	1	100.0000%	Nucleocapsid	S235F
SNP	g28980t	N/A	1	100.0000%	Nucleocapsid	G236V

¹Coverage for all variants in Table II 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 (EPI_ISL_1055380) 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 II 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.