

**SARS-Related Coronavirus 2, Isolate hCoV-19/USA/NY-NP-DOH1/2021 (Lineage B.1.526)**

**Catalog No. NR-55359**

**Product Description:**

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/USA/NY-NP-DOH1/2021 was isolated from a nasopharyngeal swab in New York City, New York, USA in February 2021. NR-55359 lot 70043342 was produced by infecting *Homo sapiens* lung adenocarcinoma 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% CO<sub>2</sub>. The cells and supernatant were spin-clarified at 1500 × g for 10 minutes at 4°C.

**Passage History:**

VE6(1)/C(1) (Centers for Disease Control and Prevention/BEI Resources); VE6 = *Cercopithecus aethiops* kidney cells; C = Calu-3 cells

**Lot: 70043342**

**Manufacturing Date: 29MAR2021**

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TEST	SPECIFICATIONS	RESULTS
<b>Identification by Infectivity in Calu-3 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/USA/NY-NP-DOH1/2021 (GISAID: EPI_ISL_1080761)	99.96% identity with SARS-CoV-2, hCoV-19/USA/NY-NP-DOH1/2021 (GISAID: EPI_ISL_1080761)
<b>Titer by TCID<sub>50</sub> Assay in Calu-3 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 APR 2021

Program Manager or designee, ATCC Federal Solutions

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**APPENDIX I: NGS Information for NR-55359 lot 70043342**

Sequence analysis using SBC v2.0 pipeline and freebayes v1.3.1 variant caller resulted in the discovery of eight SNPs when compared to the reference sequence from GISAID EPI\_ISL\_1080761 (see Table I below). Additionally, both the reference sequence EPI\_ISL\_1080761 and NR-55359 lot 70043342 contained seventeen SNPs and one deletion (DEL) when compared to GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) (see Table II below). There were also thirteen variants referred to as ambiguous variants (AMB) in which the nucleotide in NR-55359 lot 70043342 and Wuhan-Hu-1 sequence (GenBank MN908947) are known but the reference sequence GISAID EPI\_ISL\_1080761 is ambiguous at that position, having a nucleotide among Y, R, W, K, M, S, B, D, H, V, or N (see Table III below). Due to the ambiguity of the reference sequence at this position, the variant type is otherwise unlikely to be determined, including whether there is any variation at all (i.e. the ambiguous nucleotide in the strain reference sequence GISAID EPI\_ISL\_1080761 may be identical to both NR-55359 lot 70043183 and the Wuhan-Hu-1 sequence GenBank MN908947). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

**Table I: Variants with different nucleotides between NR-55359 lot 70043342 and reference sequence EPI\_ISL\_1080761**

Variant Type	Variant Position and Identified Alternative Base	Coverage	Quality	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	t1963a	683	336.0	1	6.1493%	ORF1ab (nsp2)	Silent mutation
SNP	t1963g	683	272.0	1	5.4173%	ORF1ab (nsp2)	Silent mutation
SNP	c11750t	2563	3,383.0	1	8.2716%	ORF1ab (nsp6)	L260F
SNP	a11898g	1243	17,029.0	1	52.0515%	ORF1ab (nsp7)	Q19R
SNP	a13131g	1150	2,730.0	1	12.6957%	ORF1ab (nsp10)	Q36R
SNP	t14679c	784	959.0	1	14.5408%	ORF1ab (nsp12)	Silent mutation
SNP	t22114c	373	186.0	1	5.0938%	Spike	Silent mutation
SNP	t22990c	2443	1,217.0	1	5.0757%	Spike	Silent mutation

**Table II: Variants with different nucleotides between NR-55359 lot 70043342 and GenBank MN908947.3 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome)**

Variant Type	Variant Position and Identified Alternative Base	Coverage	Quality	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	c241t	1486	49,314.0	1	98.7214%	5'UTR	Untranslated region
SNP	c1059t	1021	35,476.0	1	99.7062%	ORF1ab (nsp2)	T85I
SNP	c3037t	738	25,988.0	1	100.0000%	ORF1ab (nsp3)	Silent mutation
SNP	t9867c	1987	49,314.0	1	99.9497%	ORF1ab (nsp4)	L438P
DEL	Δ11288-11296	961	29,402.9	-9	99.8959%	ORF1ab (nsp6)	ΔSGF (aa106-108)
SNP	c14408t	629	22,309.0	1	99.2051%	ORF1ab (nsp12)	P323L
SNP	a16500c	1985	49,314.0	1	100.0000%	ORF1ab (nsp13)	Q88H
SNP	a20262g	452	16,223.0	1	100.0000%	ORF1ab (nsp15)	Silent mutation
SNP	c21575t	5586	49,314.0	1	98.2635%	Spike	L5F
SNP	c21846t	595	20,978.0	1	100.0000%	Spike	T95I
SNP	a22320g	1444	49,314.0	1	100.0000%	Spike	D253G
SNP	g23012a	2349	49,314.0	1	99.8297%	Spike	E484K
SNP	a23403g	2055	49,314.0	1	99.7080%	Spike	D614G
SNP	c23664t	3504	49,314.0	1	100.0000%	Spike	A701V
SNP	g25563t	1679	49,314.0	1	100.0000%	ORF3a	Q57H
SNP	c28869t	3378	49,314.0	1	99.8816%	Nucleocapsid	P199L
SNP	g28975a	3106	49,314.0	1	100.0000%	Nucleocapsid	M234I
SNP	c29586t	3383	49,314.0	1	99.6157%	ORF10	P10L

Table III: Ambiguous variants with known nucleotides in NR-55359 lot 70043342 and GenBank MN908947.3 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) and ambiguous nucleotide in reference sequence EPI\_ISL\_1080761

Variant Type	Variant Position and Identified Alternative Base	Coverage	Quality	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
AMB	g1130n_g <sup>1</sup>	983	30,300.0	1	100.0000%	ORF1ab (nsp2)	Silent mutation
AMB	a1131n_a	983	30,300.0	1	100.0000%	ORF1ab (nsp2)	Silent mutation
AMB	a1255n_a	2589	81,323.3	1	100.0000%	ORF1ab (nsp2)	Silent mutation
AMB	t1256n_t	2589	81,323.3	1	100.0000%	ORF1ab (nsp2)	Silent mutation
AMB	c17845n_c	2718	84,274.2	1	99.8896%	ORF1ab (nsp13)	Silent mutation
AMB	c19484n_c	847	25,247.9	1	99.7639%	ORF1ab (nsp14)	Silent mutation
AMB	t21546n_t	45	891.4	1	100.0000%	ORF1ab (nsp16)	Silent mutation
AMB	c24981n_c	2710	82,942.5	1	99.9262%	Spike	Silent mutation
AMB	t24982n_t	2710	82,942.5	1	99.9262%	Spike	Silent mutation
AMB	c25517n_t	1041	32,955.5	1	99.9039%	ORF3a	P42L
AMB	c27925n_t	4523	142,519.0	1	100.0000%	ORF8	T11I
AMB	t28270n_t	867	27,936.5	1	99.8847%	Intergenic (ORF8/N)	Untranslated region
AMB	a28271n_t	5223	167,861.0	1	99.9234%	Intergenic (ORF8/N)	Untranslated region

<sup>1</sup>g1130n\_g represents the original nucleotide of 'g' in GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) and the ambiguous nucleotide 'n' in reference sequence GISAID EPI\_ISL\_1080761. \_g represents the nucleotide 'g' observed in NR-55359 lot 70043342.