

SARS-Related Coronavirus 2, Isolate hCoV-19/mouse/USA/IA-N501Y-MA30/2021 (Lineage B)

Catalog No. NR-56222

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate hCoV-19/mouse/USA/IA-N501Y-MA30/2021 was mouse adapted by 30 passages in mouse lung homogenate in Johnson County, Iowa, USA. NR-56222 lot 70054828 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 4 days at 37°C with 5% CO₂. The cells and supernatant were spinclarified at 1500 × g for 10 minutes at 4°C.

Passage History:

Mouse lung homogenate(30), VT(1)/C(1) (University of Iowa/BEI Resources); VT = *Cercopithecus aethiops* kidney cells with transmembrane protease, serine 2 gene (Vero E6-TMPRSS2); C = Calu-3

Lot: 70054828

Manufacturing Date: 04OCT2022

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 isolate hCoV-19/mouse/USA/IA-N501Y-MA30/2021 (GISAID: EPI_ISL_1666328)	99.99% identity with isolate hCoV-19/mouse/USA/IA-N501Y-MA30/2021 (GISAID: EPI_ISL_1666328)
Titer by TCID₅₀ Assay in Calu-3 Cells by PCR¹ (7 days at 37°C and 5% CO ₂)	Report results	2.8 × 10 ³ TCID ₅₀ per mL
Sterility (21-day incubation) Harpo's HTYE broth, 37°C and 26°C, aerobic ² Trypticase Soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Blood agar, 37°C, aerobic Blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic DMEM with 10% FBS, 37°C and 5% CO ₂	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.

²Atlas, 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-56222 lot 70054828

Sequence analysis using AMGP readsQC-illumina.py pipeline and variant caller LoFreq version: 2.1.5 resulted in the discovery of four SNPs when compared to GISAID EPI_ISL_1666328 (see Table I below). Additionally, both the reference sequence EPI_ISL_1666328 and NR-56222 lot 70054828 contained eleven 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-56222 lot 70054828 and reference sequence EPI_ISL_1666328

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	t4509c	64	1	6.2500%	ORF1ab (nsp3)	V597A
SNP	t14679c	105	1	5.7143%	ORF1ab (nsp12)	Silent mutation
SNP	t21477c	68	1	5.8824%	ORF1ab (nsp16)	Silent mutation
SNP	c26934a	125	1	6.4000%	Membrane	L138I

Table II: Variants with different nucleotides between NR-56222 lot 70054828 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	c9438t	N/A	1	100.0000%	ORF1ab (nsp4)	T295I
SNP	c12318t	N/A	1	100.0000%	ORF1ab (nsp8)	S76F
SNP	a12884g	N/A	1	100.0000%	ORF1ab (nsp9)	T67A
SNP	a20085g	N/A	1	100.0000%	ORF1ab (nsp15)	Silent mutation
SNP	a22812t	N/A	1	100.0000%	Spike	K417M
SNP	g23012a	N/A	1	100.0000%	Spike	E484K
SNP	a23040g	N/A	1	100.0000%	Spike	Q493R
SNP	a23055g	N/A	1	100.0000%	Spike	Q498R
SNP	a23063t	N/A	1	100.0000%	Spike	N501Y
SNP	a26508g	N/A	1	100.0000%	Intergenic (Envelope/Membrane)	Untranslated
SNP	g26840c	N/A	1	100.0000%	Membrane	Silent mutation

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 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.