

SARS-Related Coronavirus 2, Mouse-Adapted (in Isolate USA-WA1/2020 Backbone), Infectious Clone (ic2019-nCoV MA) in Calu-3 Cells

Catalog No. NR-55328

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate MA was developed by reverse engineering SARS-CoV-2, isolate USA-WA1/2020 for efficient interaction between viral spike protein and the mouse orthologue of the human receptor, angiotensin-converting enzyme 2 (ACE2). NR-55328 lot 70043183 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.

Passage History:

VE(1)/C(1) (University of North Carolina, Chapel Hill/BEI Resources); VE = Vero E6 cells; C = Calu-3 cells

Lot: 70043183

Manufacturing Date: 20MAR2021

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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, MA (GenBank: MT844088.1)	99.97% identity with SARS-CoV-2, MA (GenBank: MT844088.1)
Titer by TCID₅₀ Assay in Calu-3 Cells by Cytopathic Effect¹ (4 days at 37°C with 5% CO ₂)	Report results	2.8 × 10 ⁵ 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 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.

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-55328 lot 70043183

Sequence analysis using SBC v2.0 pipeline and freebayes v1.3.1 variant caller resulted in the discovery of six SNPs and one deletion (Indel) when compared to the reference sequence GenBank MT844088.1 (see Table I below). Additionally, both the reference sequence MT844088.1 and NR-55328 lot 70043183 contained six SNPs when compared to GenBank MN908947.3 (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-55328 lot 70043183 and reference sequence MT844088.1

Variant Type	Variant Position and Identified Alternative Base	Coverage	Quality	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	a5212g	4411	17218	1	16.8896%	ORF1ab (nsp3)	Silent mutation
SNP	a6721t	2235	49314	1	71.4541%	ORF1ab (nsp3)	Silent mutation
SNP	t15102a_reversion_t ¹	3532	49314	1	99.4337%	ORF1ab (nsp12)	Silent mutation
SNP	c18582a	4129	19176	1	20.2713%	ORF1ab (nsp14)	Silent mutation
SNP	t26106a	2377	10637	1	17.6273%	ORF3a	D238E
Indel	Δ27236-27237	5123	74089.8	-2	57.9725%	ORF6	A12Gfs*22 ²
SNP	a29871g	103	398	1	30.0971%	3'UTR	Untranslated region

¹t15102a represents the original mutation from "t" in GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) to "a" in the reference sequence MT844088.1. a_reversion_t represents the reversion back to t observed in NR-55328 lot 70043183. This variant is not represented as a15102t to avoid confusion regarding the wild-type nucleotide.

²The mutant protein encoded by the NR-55328 lot 70043183 ORF6 gene was aligned with the wild-type protein encoded by the SARS-CoV-2, isolate Wuhan-Hu-1 ORF6 (NC_045512). The effect of the Δ27236-27237 mutation is to introduce a -2 nucleotide frameshift, which results in a truncated protein of 21 amino acids (34.43% of the wild-type length). The proteins remain identical through amino acids 11. The first amino acid changed in NR-55328 lot 70043183 is A12G. The frameshift results in the introduction of a STOP codon at amino acid 22, with most of the amino acids between 12 to 21 mutated.

Table II: Variants with different nucleotides between NR-55328 lot 70043183 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	c18060t	3511	49314	1	99.7437%	ORF1ab (nsp14)	Silent mutation
SNP	c23054t	2410	49314	1	98.7552%	Spike	Q498Y
SNP	a23056c	2347	49314	1	98.8070%		
SNP	c23057a	2347	49314	1	98.8070%	Spike	P499T
SNP	c23059g	2436	49314	1	99.8358%		
SNP	t28144c	8749	49314	1	99.8585%	ORF8	L84S