

SARS-Related Coronavirus 2, Mouse-Adapted, MA10 Variant (in isolate USA-WA1/2020 backbone), Infectious Clone (ic2019-nCoV MA10) in Calu-3 Cells

Catalog No. NR-55329

Product Description:

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate MA10 was developed by ten *in vivo* serial passages of SARS-CoV-2, isolate MA in BALB/c mice, followed by plaque purification in *Cercopithecus aethiops* kidney epithelial cells (Vero E6). Following deep sequencing of the plaque purified virus, an infectious clone was generated for subsequent preparation of the MA10 virus stock. NR-55329 lot 70043185 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: 70043185

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, MA10 (GenBank: MT952602.1)	99.96% identity with SARS-CoV-2, MA10 (GenBank: MT952602.1)
Titer by TCID₅₀ Assay in Calu-3 Cells by Cytopathic Effect¹ (4 days at 37°C with 5% CO ₂)	Report results	1.6 × 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-55329 lot 70043185

Sequence analysis using SBC v2.0 pipeline and freebayes v1.3.1 variant caller resulted in the discovery of fourteen SNPs when compared to the reference sequence GenBank MT952602.1 (see Table I below). Additionally, both the reference sequence MT952602.1 and NR-55329 lot 70043185 contained eleven 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-55329 lot 70043185 and reference sequence MT952602.1

Variant Type	Variant Position and Identified Alternative Base	Coverage	Quality	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	c3768a	1328	2037	1	7.8313%	ORF1ab (nsp3)	T350N
SNP	c6926t	2456	3242	1	7.8583%	ORF1ab (nsp3)	P1403S
SNP	t10362g	2933	7543	1	11.7968%	ORF1ab (nsp5)	F103C
SNP	t15102a_reversion_t ¹	3499	49314	1	99.3998%	ORF1ab (nsp12)	Silent mutation
SNP	c15368a	2959	4275	1	7.2998%	ORF1ab (nsp12)	T634K
SNP	g16696a	2982	3756	1	6.4722%	ORF1ab (nsp13)	V154I
SNP	c17550g	3589	3523	1	7.1329%	ORF1ab (nsp13)	Silent mutation
SNP	g17887a	2114	1681	1	5.0615%	ORF1ab (nsp13)	E551K
SNP	c19186a	2089	1913	1	5.9359%	ORF1ab (nsp14)	L383I
SNP	c23657a	2905	5038	1	8.9157%	Spike	L699I
SNP	c25771a	3167	3616	1	7.1045%	ORF3a	L127I
SNP	a27003c	3549	5371	1	7.7768%	M (Membrane protein)	I161L
SNP	t28912a	4430	12319	1	12.5734%	N (Nucleocapsid)	N213K
SNP	a29871g	72	253	1	27.7778%	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 MT952602.1. a_reversion_t represents the reversion back to t observed in NR-55329 lot 70043185. This variant is not represented as a15102t to avoid confusion regarding the wild-type nucleotide.

Table II: Variants with different nucleotides between NR-55329 lot 70043185 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	c9438t	1435	49314	1	99.7909%	ORF1ab (nsp4)	T295I
SNP	a11847g	2603	49314	1	99.8079%	ORF1ab (nsp7)	K2R
SNP	a12159g	2976	49314	1	99.7312%	ORF1ab (nsp8)	E23G
SNP	c18060t	3544	49314	1	99.8871%	ORF1ab (nsp14)	Silent mutation
SNP	c23039a	2894	49314	1	99.8272%	Spike	Q493K
SNP	c23054t	2477	49314	1	98.9100%	Spike	Q498Y
SNP	a23056c	2419	49314	1	98.9665%	Spike	Q498Y
SNP	c23057a	2419	49314	1	98.9252%	Spike	P499T
SNP	c23059g	2473	49314	1	99.6765%	Spike	P499T
SNP	t27221c	3611	49314	1	99.8061%	ORF6	F7S
SNP	t28144c	8371	49314	1	99.8805%	ORF8	L84S