

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

Catalog No. NR-59317

## 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-59317 lot 70059895 was produced by infecting *Homo sapiens* lung adenocarcinoma epithelial cells (Calu-3; ATCC® HTB-55™) with seed material (BEI Resources lot 70041092) and incubating in Eagle's Minimum Essential Medium (ATCC® 30-2003™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) for 7 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.

Total Passage: 3

## Passage History:

VE(1)/VE(1),C(1) (University of North Carolina, Chapel Hill/BEI Resources /BEI Resources); VE = Vero E6 cells, C = *Homo sapiens* lung adenocarcinoma epithelial cells (Calu-3)

Lot: 70059895

Manufacturing Date: 05APR2023

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)	99.98% identity with SARS-CoV-2, MA10 (GenBank: MT952602)
Titer by TCID <sub>50</sub> Assay in Calu-3 Cells by CPE <sup>1,2</sup> (10 days at 37°C and 5% CO <sub>2</sub> )	Report results	1.2 × 10 <sup>4</sup> TCID <sub>50</sub> per mL
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 <sup>3</sup> 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 <sub>2</sub>	No growth No growth No growth No growth No growth No growth No growth	Pending Pending Pending Pending Pending Pending Pending
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	Pending Pending

<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>Titer was determined by cytopathic effects (CPE) and completed in triplicate (1.6 × 10<sup>4</sup> per mL, 1.6 × 10<sup>4</sup> per mL and 2.8 × 10<sup>3</sup> per mL). The average of the three values is reported.

<sup>3</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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## APPENDIX I: NGS Information for NR-59317 lot 70059895

Sequence analysis using AMGP readsQC-illumina.py pipeline and variant caller LoFreq version: 2.1.5 resulted in the discovery of six SNPs when compared to GenBank MT952602 (see Table I below). Additionally, both the reference sequence MT952602 and NR-59317 lot 70059895 contained eleven SNPs and one DEL when compared to GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) (see Table II below). The reference sequence MT952602 has one SNP when compared to both NR-59317 lot 70059895 and GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) (see Table III below). Quality scores over 60 indicate it is improbable that the variant call is incorrect.

**Table I: Variants with different nucleotides between NR-59317 lot 70059895 and reference sequence MT952602**

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	a10620g	216	1	5.0926%	ORF1ab (nsp5)	Q189R
SNP	t11963c	325	1	5.8462%	ORF1ab (nsp7)	Silent mutation
SNP	c16394g	224	1	10.2679%	ORF1ab (nsp13)	P53R
SNP	c23606g	205	1	9.2683%	Spike	R682G
SNP	a27486g	218	1	6.4220%	ORF7a	Silent mutation
SNP	c28869a	254	1	6.2992%	Nucleocapsid	P199Q

**Table II: Variants with different nucleotides between NR-59317 lot 70059895 and GenBank MN908947.3 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome)**

Variant Type	Variant Position and Identified Alternative Base	Coverage <sup>1</sup>	Length of Variant	Frequency of Variant <sup>2</sup>	Gene (Region)	Amino Acid Mutation
SNP	c9438t	N/A	1	100.0000%	ORF1ab (nsp4)	T295I
SNP	a11847g	N/A	1	100.0000%	ORF1ab (nsp7)	K2R
SNP	a12159g	N/A	1	100.0000%	ORF1ab (nsp8)	E23G
SNP	c18060t	N/A	1	100.0000%	ORF1ab (nsp14)	Silent mutation
SNP	c23039a	N/A	1	100.0000%	Spike	Q493K
SNP	c23054t	N/A	1	100.0000%	Spike	Q498Y
SNP	a23056c	N/A	1	100.0000%		
SNP	c23057a	N/A	1	100.0000%	Spike	P499T
SNP	c23059g	N/A	1	100.0000%		
SNP	t27221c	N/A	1	100.0000%	ORF6	F7S
SNP	t28144c	N/A	1	100.0000%	ORF8	L84S
DEL	29902	N/A	-1	100.0000%	3'UTR	Untranslated

# Certificate of Analysis for NR-59317

<sup>1</sup>Coverage 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 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.

**Table III: Variant with nucleotide difference between both NR-59317 lot 70059895 and GenBank MN908947.3 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) and reference sequence MT95260**

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	t15102a_rev_t <sup>1</sup>	246	1	99.5935%	ORF1ab (nsp12)	Silent mutation

<sup>1</sup>rev = reversion: t15102a\_rev\_t represents the original mutation from "t" in Wuhan-Hu-1 to "a" in the provided reference genome and the reversion back to t observed in the sample. (This variant is not represented as a15102t to avoid confusion regarding the wild-type nucleotide.)