Certificate of Analysis for NR-60440

SARS-Related Coronavirus 2, Mouse-Adapted, MA10 Variant (in Isolate USA-WA1/2020 Backbone), Infectious Clone (ic2019-nCoV MA10) in VTA Cells

Catalog No. NR-60440

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 *Chlorocebus aethiops (C. 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-60440 was produced by infecting *C. aethiops* kidney epithelial cells expressing transmembrane protease, serine 2 and human angiotensin-converting enzyme 2 (Vero E6-TMPRSS2-T2A-ACE2; VTA; BEI Resources NR-54970) with BEI Resources seed lot 70043186 and incubating in Eagle's Minimum Essential Medium containing Earle's Balanced Salt Solution, non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate and 1.5 g/L of sodium bicarbonate (ATCC® 30-2003™), supplemented with 2% fetal bovine serum (ATCC® 30-2020™) for 3 days at 37°C in an aerobic atmosphere with 5% CO_2. The cells and supernatant were spin-clarified at 1500 × g for 10 minutes at 4°C.

Passage History:

VE(1)/C(1)VTA(1) (University of North Carolina, Chapel Hill/BEI Resources); VE = Vero E6 cells; C = Calu-3 cells; VTA = *C. aethiops* kidney cells expressing transmembrane protease, serine 2 gene and human angiotensin-converting enzyme 2 (Vero E6-TMPRSS2-T2A-ACE2)

Lot: 70075063 Manufacturing Date: 31MAR2025

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TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity in VTA Cells	Syncytia formation and sloughing	Syncytia formation and sloughing
Next-Generation Sequencing (NGS) of Complete Genome Using Illumina® MiSeq™ Platform (Refer to Appendix I for NGS information)	≥ 98% sequence identity with SARS-CoV-2, MA10 (GenBank: MT952602.1)	99.95% sequence identity with SARS-CoV-2, MA10 (GenBank: MT952602.1)
Titer by $TCID_{50}$ Assay in VTA Cells by Cytopathic Effect[1,2] (6 days at 37°C and 5% CO_2)	Report results	7.2×10^6 $TCID_{50}$ per mL
Endotoxin Content (*Limulus* Amoebocyte Lysate Assay)	Report results	≤ 0.03 EU/mL
Sterility (21-day incubation)		
Harpo's HTYE broth, 37°C and 26°C, aerobic[3]	No growth	Pending
Trypticase Soy broth, 37°C and 26°C, aerobic	No growth	Pending
Sabouraud broth, 37°C and 26°C, aerobic	No growth	Pending
Blood agar, 37°C, aerobic	No growth	Pending
Blood agar, 37°C, anaerobic	No growth	Pending
Thioglycollate broth, 37°C, anaerobic	No growth	Pending
DMEM with 10% FBS, 37°C and 5% CO_2	No growth	Pending
Mycoplasma Contamination		
Agar and broth culture (14-day incubation at 37°C)	None detected	Pending
DNA detection by PCR of extracted Test Article nucleic acid	None detected	None detected

[1]The Tissue Culture Infectious Dose 50% ($TCID_{50}$) endpoint is the 50% infectious endpoint in cell culture. The $TCID_{50}$ 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_{50}) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the $TCID_{50}$ provides a measure of the titer (or infectivity) of a virus preparation.
[2]Titer was determined by cytopathic effects (CPE) and completed in triplicate (1.6×10^7 $TCID_{50}$/mL, 2.8×10^6 $TCID_{50}$/mL and 2.8×10^6 $TCID_{50}$/mL). The average of the three values is reported.
[3]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

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Technical Manager or designee, ATCC Federal Solutions

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

Support Provided by NIAID

APPENDIX I: NGS INFORMATION for NR-60440 lot 70075063

Sequence analysis using AMGP readsQC-illumina.py pipeline and variant caller LoFreq version: 2.1.5 resulted in the discovery of fourteen SNPs and one INS when compared to reference sequence GenBank: MT952602.1 (SARS-CoV-2, MA10) (see Table I below). Additionally, both the reference sequence GenBank: MT952602.1 and NR-60440 lot 70075063 contained eleven SNPs and one DEL when compared to GenBank MN908947.3 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) (see Table II below). Finally, NR-60440 lot 70075063 and MN908947.3 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome) contained one SNP when compared to GenBank MT952602.1 (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-60440 lot 70075063 and reference sequence GenBank: MT952602.1

Variant Type	Variant Position and Identified Alternative Base	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	c6926t	1734	1	15.7439%	ORF1ab (nsp3)	P1403S
SNP	a8307g	2315	1	8.8121%	ORF1ab (nsp3)	E1863G
SNP	t10362g	2248	1	20.5961%	ORF1ab (nsp5)	F103C
SNP	t14679c	1354	1	9.8227%	ORF1ab (nsp12)	Silent mutation
SNP	c15368a	1490	1	16.7114%	ORF1ab (nsp12)	T643K
SNP	c17550g	1966	1	16.9379%	ORF1ab (nsp13)	Silent mutation
SNP	g17887a	1510	1	14.8344%	ORF1ab (nsp13)	E551K
SNP	c24986a	3177	1	6.6415%	Spike	Q1142K
SNP	c26299a	2138	1	8.6529%	Envelope	L19I
SNP	a26855g	2495	1	7.2545%	Membrane	Silent mutation
SNP	a27003c	2440	1	5.1639%	Membrane	I161L
INS	27204[t]27205	1915	+1	19.2167%	ORF6	MFHLVDFQ VTIAEILLIIM RTFKVSIWN LDYIINLIIKNL SKSLTENKYS QLDEEQPME ID*→MFSSR*
SNP	t28912a	3107	1	22.8516%	Nucleocapsid	N213K
SNP	a29839g	1487	1	7.2629%	3'UTR	Untranslated
SNP	a29871g	123	1	21.9512	3'UTR	Untranslated

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Table II: Variants with different nucleotides between NR-60440 lot 70075063 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	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	Q498*
SNP	a23056c	N/A	1	100.0000%	Spike	P499T
SNP	c23057a	N/A	1	100.0000%		
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	Δ29902	N/A	-1	100.0000%	3'UTR	Untranslated

[1]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 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.

Table III: Variants with different nucleotides between NR-60440 lot 70075063 and reference sequence GenBank: MT952602.1, but matching nucleotides with GenBank MN908947.3 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome)

Variant Type	Variant Position and Identified Alternative Base[2]	Coverage	Length of Variant	Frequency of Variant	Gene (Region)	Amino Acid Mutation
SNP	t15102a_rev_t	2079	1	99.1823%	ORF1ab (nsp12)	Silent mutation

[2]rev = reversion. For example, "t15102a" represents the original mutation from "t" in Wuhan-Hu-1 to "a" in the provided reference genome. "_rev_t" represents the reversion back to "t" observed in the sample. (This variant is not represented as "t15102a" to avoid confusion regarding the wild-type nucleotide.)

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