

**SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Recombinant Infectious Clone with Nanoluciferase Gene (icSARS-CoV-2-nLuc)**

**Catalog No. NR-54003**

**Product Description:**

NR-54003 is a recombinant infectious clone of severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), USA-WA1/2020 with nanoluciferase (nLuc) gene (icSARS-CoV-2-nLuc). NR-54003 lot 70041552 was produced by infecting human lung adenocarcinoma cells (Calu-3; ATCC® HTB-55™) with the deposited material in Eagle's Minimum Essential Medium (ATCC® 30-2003™) supplemented with 2% fetal bovine serum (ATCC® 30-2020™) for 5 days at 37°C with 5% CO<sub>2</sub>.

**Passage History:**

V(1)/C(1) (Prior to deposit at BEI Resources/BEI Resources); V = Vero; C = Calu-3 cells

**Lot: 70041552**

**Manufacturing Date: 18JAN2021**

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TEST	SPECIFICATIONS	RESULTS
<b>Identification by Infectivity in Calu-3 Cells</b>	Cell rounding and detachment	Cell rounding and detachment
<b>Next-Generation Sequencing (NGS) of Complete Genome Using Illumina® iSeq™ 100 Platform</b> (Refer to Appendix I for NGS information)	≥ 98% identity with synthetic construct clone icSARS-CoV-2-nLuc-GFP (GenBank: MT461671.1) nLuc sequence confirmed	99.96% identity with synthetic construct clone icSARS-CoV-2-nLuc-GFP (GenBank: MT461671.1) nLuc sequence confirmed <sup>1</sup>
<b>Titer by TCID<sub>50</sub> Assay in Calu-3 Cells by Cytopathic Effect<sup>2</sup></b> (6 days at 37°C and 5% CO <sub>2</sub> )	Report results	2.8 × 10 <sup>4</sup> TCID <sub>50</sub> per mL
<b>Sterility (21-day incubation)</b> 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 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
<b>Mycoplasma Contamination</b> 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

<sup>1</sup>Expression of nanoluciferase was not determined at BEI Resources. It is recommended to confirm nanoluciferase expression prior to initiating work.

<sup>2</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>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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16 JUN 2021

Program Manager or designee, ATCC Federal Solutions

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**APPENDIX I: NGS Information for NR-54003 lot 70041552**

Sequence analysis using SBC v2.0 pipeline resulted in the discovery of fourteen SNPs when compared to the reference sequence GenBank MT461671.1 (see Table I below). Additionally, both the reference sequence MT461671.1 and NR-54003 lot 70041552 contained three 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-54003 lot 70041552 and reference sequence MT461671.1**

Position in NR-54003_70041552 Sequence	Position in MN908947 Wuhan-Hu-1 Sequence	Position in MT461671 Reference Sequence	Reported MN908947 Wuhan-Hu-1 Sequence	Reported MT461671 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
1959	1963	1963	T	T	G	335	SNP	1	0.053915
8778	8782	8782	C	T	C	42230	SNP	1	0.998467
14675	14679	14679	T	T	C	1374	SNP	1	0.118147
15905	15909	15909	T	T	C	600	SNP	1	0.051917
19899	19903	19903	C	C	A	15151	SNP	1	0.362803
20462	20466	20466	T	T	A	4787	SNP	1	0.283862
22110	22114	22114	T	T	C	387	SNP	1	0.063861
23609	23613	23613	C	C	A	11617	SNP	1	0.287438
23614	23618	23618	A	A	G	3568	SNP	1	0.104494
24792	24796	24796	T	T	C	10268	SNP	1	0.298246
25802	25806	25806	A	A	G	534	SNP	1	0.060669
27426	27430	27430	G	A	G	430	SNP	1	1.000000
28142	N/A <sup>1</sup>	28146	N/A <sup>1</sup>	G	C	316	SNP	1	0.809524
30789	29839	30793	A	A	G	201	SNP	1	0.064202

<sup>1</sup>N/A is reported for the position and nucleotide of the SARS-CoV-2, Wuhan-Hu-1 isolate (GenBank: MN908947) because this position lies in the nanoluciferase gene and there is no corresponding nucleotide for Wuhan-Hu-1 isolate at that position.

**Table II: Variants with different nucleotides between NR-54003 lot 70041552 and GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome)**

Position in NR-54003_70041552 Sequence	Position in MN908947 Wuhan-Hu-1 Sequence	Position in MT461671 Reference Sequence	Reported MN908947 Wuhan-Hu-1 Sequence	Reported MT461671 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
15098	15102	15102	T	A	A	N/A	SNP	1	1.000000
18056	18060	18060	C	T	T	N/A	SNP	1	1.000000
29094	28144	29098	T	C	C	48559	SNP	1	1.000000