

SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Recombinant Infectious Clone with Enhanced Green Fluorescent Protein (icSARS-CoV-2-eGFP)

Catalog No. NR-54002

Product Description:

NR-54002 is a recombinant infectious clone (icSARS-CoV-2-eGFP) of severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), USA-WA1/2020 with enhanced green fluorescent protein (eGFP). NR-54002 lot 70041550 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% CO₂.

Passage History:

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

Lot: 70041550

Manufacturing Date: 18JAN2021

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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 synthetic construct clone icSARS-CoV-2-GFP (GenBank: MT461670.1) eGFP sequence confirmed	99.96% identity with synthetic construct clone icSARS-CoV-2-GFP (GenBank: MT461670.1) eGFP sequence confirmed
Titer by TCID₅₀ Assay in Calu-3 Cells by Cytopathic Effect¹ (6 days at 37°C and 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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15 JAN 2023

Technical Manager or designee, ATCC Federal Solutions

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APPENDIX I: NGS Information for NR-54002 lot 70041550

Sequence analysis using SBC v2.0 pipeline resulted in the discovery of eleven SNPs when compared to the reference sequence GenBank MT461670.1 (see Table I below). Additionally, both the reference sequence MT461670.1 and NR-54002 lot 70041550 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-54002 lot 70041550 and reference sequence MT461670.1

Position in NR-54002_70041550 Sequence	Position in MN908947 Wuhan-Hu-1 Sequence	Position in MT461670 Reference Sequence	Reported MN908947 Wuhan-Hu-1 Sequence	Reported MT461670 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
6441	6445	6445	C	C	A	2783	SNP	1	0.058161
7726	7730	7730	T	T	C	2898	SNP	1	0.099628
8778	8782	8782	C	T	C	49314	SNP	1	1.000000
16565	16569	16569	A	A	C	17510	SNP	1	0.206525
18080	18084	18084	C	C	A	5334	SNP	1	0.067536
19574	19578	19578	A	A	G	5843	SNP	1	0.076597
22110	22114	22114	T	T	C	781	SNP	1	0.059303
27426	27430	27430	A	A	C	3935	SNP	1	0.077866
29217	28777	29221	A	A	C	27982	SNP	1	0.359234
29231	28791	29235	C	C	T	31895	SNP	1	0.375774
30279	29839	30283	A	A	G	498	SNP	1	0.069470

Table II: Variants with different nucleotides between NR-54002 lot 70041550 and GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome)

Position in NR-54002_70041550 Sequence	Position in MN908947 Wuhan-Hu-1 Sequence	Position in MT461670 Reference Sequence	Reported MN908947 Wuhan-Hu-1 Sequence	Reported MT461670 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
28584	28144	28588	T	C	C	N/A	SNP	1	1.000000