

SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Recombinant Infectious Clone (icSARS-CoV-2-WT)

Catalog No. NR-54001

Product Description:

NR-54001 is a recombinant infectious cDNA clone (icSARS-CoV-2-WT) of severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate USA-WA1/2020. **Note: NR-54001 label lists this clone as wildtype (WT); however, NR-54001 is not WT and includes a T15102A silent mutation.** NR-54001 lot 70041548 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: 70041548

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-WT (GenBank: MT461669.1)	99.96% identity with synthetic construct clone icSARS-CoV-2-WT (GenBank: MT461669.1)
Titer by TCID ₅₀ Assay in Calu-3 Cells by Cytopathic Effect ¹ (5 days at 37°C and 5% CO ₂)	Report results	2.8 × 10 ⁴ 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 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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16 JUN 2021

Program Manager or designee, ATCC Federal Solutions

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APPENDIX I: NGS Information for NR-54001 lot 70041548

Sequence analysis using SBC v2.0 pipeline resulted in the discovery of eleven SNPs when compared to the reference sequence GenBank MT461669.1 (see Table I below). Additionally, both the reference sequence MT461669.1 and NR-54001 lot 70041548 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-54001 lot 70041548 and reference sequence MT461669.1

Position in NR-54001_70041548 Sequence	Position in MN908947 Wuhan-Hu-1 Sequence	Position in MT461669 Reference Sequence	Reported MN908947 Wuhan-Hu-1 Sequence	Reported MT461669 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
8771	8782	8782	C	T	C	45868	SNP	1	1.000000
14566	14577	14577	T	T	A	8630	SNP	1	0.153876
16053	16064	16064	A	A	C	1267	SNP	1	0.050161
19723	19734	19734	T	T	G	5928	SNP	1	0.146205
20211	20222	20222	A	A	C	7076	SNP	1	0.152614
22103	22114	22114	T	T	C	389	SNP	1	0.058224
22290	22301	22301	A	A	C	3408	SNP	1	0.104343
24938	24949	24949	A	A	G	1701	SNP	1	0.050839
26256	26267	26267	A	A	T	15437	SNP	1	0.324007
28242	28253	28253	C	C	T	6909	SNP	1	0.152501
29828	29839	29839	A	A	G	203	SNP	1	0.053691

Table II: Variants with different nucleotides between NR-54001 lot 70041548 and GenBank MN908947 (SARS-CoV-2, isolate Wuhan-Hu-1, complete genome)

Position in NR-54001_70041548 Sequence	Position in MN908947 Wuhan-Hu-1 Sequence	Position in MT461669 Reference Sequence	Reported MN908947 Wuhan-Hu-1 Sequence	Reported MT461669 Reference Sequence	Identified Alternative Base	Quality	Variant Type	Length of Variant	Frequency of Variant
15091	15102	15102	T	A	A	N/A	SNP	1	1.000000
18059	18060	18060	C	T	T	N/A	SNP	1	1.000000
28133	28144	28144	T	C	C	N/A	SNP	1	1.000000