

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

Catalog No. NR-54001

For research use only. Not for use in humans.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

Note: NR-54001 label lists this clone as wildtype (WT); however, NR-54001 is not WT and includes a T15102A silent mutation.

Virus Classification: *Coronaviridae*, *Betacoronavirus*

Species: Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2)

Isolate: Recombinant infectious clone of SARS-CoV-2, USA-WA1/2020 (icSARS-CoV-2-WT)^{1,2}

Original Source: NR-54001 is an infectious complementary DNA (cDNA) clone of SARS-CoV-2, isolate USA-WA1/2020 created using a reverse genetics system.^{1,2}

Note: Genome sequence information is provided on the Certificate of Analysis and includes an analysis of all sequence variations observed for each lot.

Comments: Full-length infectious cDNA of SARS-CoV-2 virus, isolate USA-WA1/2020 was generated by cloning seven genomic fragments separately into vector plasmids. A silent mutation (T15102A) to ablate an endogenous *SacI* site was created in a conserved region of the non-structural protein 12 to distinguish the infectious clone from circulating virus.² After assembly into full-length cDNA, full-length RNA was generated and electroporated into *Cercopithecus aethiops* kidney epithelial cells (Vero E6) to recover icSARS-CoV-2-WT virus. Replication of the recombinant virus icSARS-CoV-2-WT was confirmed, and the virus could be successfully passaged serially in cell culture to titers equivalent to the clinical isolate.² The complete genome of recombinant virus icSARS-CoV-2-WT has been sequenced (GenBank: [MT461669](https://www.ncbi.nlm.nih.gov/nuclot/MT461669)).

In December 2019, an outbreak of a respiratory illness (COVID-19) began in Wuhan, Hubei Province, China. The outbreak is associated with a seafood market and although environmental samples from the market are positive for the novel coronavirus, an association with a particular animal has not been determined.³

Material Provided:

Each vial contains approximately 0.3 mL of spin-clarified cell lysate and supernatant from *Homo sapiens* lung adenocarcinoma cells infected with icSARS-CoV-2-WT.

Note: If homogeneity is required for your intended use, please purify prior to initiating work.

Packaging/Storage:

NR-54001 was packaged aseptically in screw-capped plastic cryovials. The product is provided frozen and should be stored at -60°C or colder immediately upon arrival. For long-term storage, the vapor phase of a liquid nitrogen freezer is recommended. Freeze-thaw cycles should be avoided.

Growth Conditions:

Host: *Homo sapiens* lung adenocarcinoma cells (Calu-3; ATCC® HTB-55™)

Growth Medium: Eagle's Minimum Essential Medium containing Earle's Balanced Salt Solution, non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate and 1500 mg per L of sodium bicarbonate supplemented with 2% fetal bovine serum, or equivalent

Infection: Cells should be 50% to 100% confluent

Incubation: 4 to 6 days at 37°C and 5% CO₂

Cytopathic Effect: Cell rounding and sloughing

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Recombinant Infectious Clone (icSARS-CoV-2-WT), NR-54001."

Biosafety Level: 3

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 6th ed. Washington, DC: U.S. Government Printing Office, 2020; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

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References:

1. Baric, R. S., Personal Communication.
2. Hou, Y. J., et al. "SARS-CoV-2 Reverse Genetics Reveals a Variable Infection Gradient in the Respiratory Tract." *Cell* 182 (2020): 429-446. PubMed: 32526206.
3. Gralinski, L. E. and V. D. Menachery. "Return of the Coronavirus: 2019-nCoV." *Viruses* 12 (2020): 135. PubMed: 31991541.

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