

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

Catalog No. NR-54003

For research use only. Not for use in humans.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

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 with nanoluciferase (nLuc) gene (icSARS-CoV-2-nLuc)

Original Source: NR-54003 is an infectious complementary DNA (cDNA) clone of SARS-CoV-2, isolate USA-WA1/2020 with nLuc gene, created using a reverse genetics system.^{1,2}

Comments: Full-length infectious cDNA of SARS-CoV-2 virus, isolate USA-WA1/2020 was generated by cloning 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 A 276 base pairs region of open reading frame 7 (ORF7) in SARS-CoV-2, isolate icSARS-CoV-2-nLuc was replaced by the gene encoding nLuc.¹ nLuc is an engineered luciferase consisting of a modified 19 kilodaltons subunit of deep sea shrimp *Oplophorus gracilirostris* luciferase, modified to generate brighter and stable glow-type luminescence.³ 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-nLuc virus.^{1,2}

Material Provided:

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

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

Packaging/Storage:

NR-54003 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: Human 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 80% 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 with Nanoluciferase Gene (icSARS-CoV-2-nLuc), NR-54003."

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/bmb15/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. Hall, M. P., et al. "Engineered Luciferase Reporter from a Deep Sea Shrimp Utilizing a Novel Imidazopyrazinone Substrate." *ACS Chem. Biol.* 7 (2012): 1848-1857. PubMed: 22894855.

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