

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

Catalog No. NR-54002

For research use only. Not for use in humans.

Contributor:

Ralph S. Baric, Ph.D., Department of Microbiology and Immunology, University of North Carolina School of Medicine, Chapel Hill, North Carolina, USA

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 enhanced green fluorescent protein (eGFP) (icSARS-CoV-2-eGFP)^{1,2}

Original Source: NR-54002 is an infectious complementary DNA (cDNA) clone of SARS-CoV-2, isolate USA-WA1/2020 with eGFP, 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 A 276 base pairs region of open reading frame 7 (ORF7) in SARS-CoV-2, isolate icSARS-CoV-2-GFP was replaced by an approximately 720 base pair enhanced green fluorescent protein (eGFP).^{1,2} eGFP is a modified version of the GFP gene designed for brighter fluorescence, in which the codon utilization has been maximized for translation in eukaryotic cells.^{3,4} 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-eGFP virus. Replication of the recombinant virus icSARS-CoV-2-eGFP 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-eGFP has been sequenced (GenBank: [MT461670](https://www.ncbi.nlm.nih.gov/nuccore/MT461670)).

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-eGFP.

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

Packaging/Storage:

NR-54002 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 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: Recombinant SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Recombinant Infectious Clone with Enhanced Green Fluorescent Protein (icSARS-CoV-2-eGFP), NR-54002."

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.

Disclaimers:

You are authorized to use this product for research use only. It is not intended for human use.

Use of this product is subject to the terms and conditions of the BEI Resources Material Transfer Agreement (MTA). The MTA is available on our Web site at www.beiresources.org.

While BEI Resources uses reasonable efforts to include accurate and up-to-date information on this product sheet, neither ATCC® nor the U.S. Government makes any warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. Neither ATCC® nor the U.S. Government warrants that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, use and disposal. ATCC® and the U.S. Government are not liable for any damages or injuries

arising from receipt and/or use of this product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, the U.S. Government, ATCC®, their suppliers and contributors to BEI Resources are not liable for damages arising from the misidentification or misrepresentation of products.

Use Restrictions:

This material is distributed for internal research, non-commercial purposes only. This material, its product or its derivatives may not be distributed to third parties. Except as performed under a U.S. Government contract, individuals contemplating commercial use of the material, its products or its derivatives must contact the contributor to determine if a license is required. U.S. Government contractors may need a license before first commercial sale.

Registrants from for-profit companies need to obtain a license from University of North Carolina at Chapel Hill before BEI Resources distributes the material. Registrants will submit the request for a license to researchtools@unc.edu and will be responsible for including this with their order request to BEI Resources.

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. Cormack, B. P., R. H. Valdivia and S. Falkow. "FACS-Optimized Mutants of the Green Fluorescent Protein (GFP)." *Gene* 173 (1996): 33-38. PubMed: 8707053.
4. Haas, J., E. C. Park and B. Seed. "Codon Usage Limitation in the Expression of HIV-1 Envelope Glycoprotein." *Curr. Biol.* 6 (1996): 315-324. PubMed: 8805248.

ATCC® is a trademark of the American Type Culture Collection.

