

pSMART® BAC V2.0 Vector Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Non-Infectious Replicon

Catalog No. NR-54972

For research use only. Not for use in humans.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

The vector for the non-infectious replicon from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: [NC_045512](#)) was designed by cloning a T7 promoter upstream of the SARS-CoV-2 insert and a poly A/HDV ribozyme and T7 terminator cassette downstream and subcloned into the [pSMART® BAC V2.0](#) cloning vector.^{1,2,3} The spike (S) gene was replaced by a luciferase (Luc2) and enhanced green fluorescent protein (eGFP) fusion construct. The Luc2 gene is a synthetic firefly luciferase gene that has been codon optimized for mammalian expression and has had cryptic transcription factor binding sites removed. The envelope (E) and membrane (M) genes were replaced with a neomycin resistance gene, aminoglycoside transferase. NR-54972 contains the chloramphenicol acetyltransferase gene, *cat*, to provide transformant selection through chloramphenicol resistance in *Escherichia coli* (*E. coli*) and a neomycin selectable marker for mammalian expression. The resulting size of the plasmid is approximately 36000 base pairs. The complete plasmid sequence and map are provided on the BEI Resources webpage. The plasmid was produced in *E. coli* and extracted.

NR-54972 is used to express a SARS-CoV-2 replicon that is defective in producing progeny virions. The genome is replication competent in multiple cell lines.²

Material Provided:

Each vial contains plasmid DNA in EB buffer (10 mM Tris-HCl, pH 8.5). The DNA concentration and volume provided are shown on the Certificate of Analysis. The vial should be centrifuged prior to opening. Note: The contents of the vial should be used to replicate the plasmid in *E. coli* prior to mammalian expression.

Packaging/Storage:

NR-54972 was packaged aseptically in screw-capped plastic cryovials. The product is provided frozen on dry ice and should be stored at -20°C or colder immediately upon arrival. Freeze-thaw cycles should be minimized.

Note: NR-54972 was not provided in ethylenediamine-tetraacetic acid (EDTA); for long-term storage, EDTA may be added to a final concentration of 1 mM.

Citation:

Acknowledgment for publications should read “The following reagent was obtained through BEI Resources, NIAID, NIH: pSMART® BAC V2.0 Vector Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Non-Infectious Replicon, NR-54972.”

Biosafety Level: 1

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.

Note: Non-infectious replicons produced by use of NR-54972 are a BSL2 organism. Replicon production should be performed with appropriate biosafety controls.

Disclaimers:

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NR-54972 is claimed in U.S. Provisional Patent Application number 63/083,852 as well as U.S. Patent number 8,008,006 and European Patent number 1341808 and the continuations, continuations-in-part, re-issues and foreign counterparts thereof.

References:

1. Wang, D., Personal Communication.
2. He, X., et al. "Generation of a SARS-CoV-2 Replicon as a Model System to Dissect Virus Replication and Antiviral Inhibition." [bioRxiv](#) (2020). doi: 10.1101/2020.12.12.422532.
3. Wu, F., et al. "A New Coronavirus Associated with Human Respiratory Disease in China." [Nature](#) 579 (2020): 265-269. PubMed: 32015508.

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