

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

Catalog No. NR-54972

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 (GenBank: [EU101022](#)). The spike (S) gene was replaced by a luciferase (Luc2) and enhanced green fluorescent protein (eGFP) fusion construct. 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. NR-54972 was produced by the inoculation of BEI Resources seed lot 70042539 into Luria-Bertani broth with chloramphenicol (12.5 µg/mL) and incubated for 20 hours at 37°C in an aerobic atmosphere to produce this lot. The plasmid was extracted using a Plasmid *Plus* Maxi Kit (QIAGEN® 12963) and vialled in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Lot: 70064038

Manufacturing Date: 08FEB2024

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	36,026 base pairs	36,024 base pairs ¹
Genotypic Analysis Sequencing of SARS-CoV-2 replicon insert (28,473 base pairs)	≥ 99% sequence identity to depositor's sequence Luc2-eGFP fusion sequence present	100% sequence identity to depositor's sequence Luc2-eGFP fusion sequence present
Antibiotic Resistance Chloramphenicol (encoded by chloramphenicol acetyltransferase gene <i>cat</i>) Neomycin [encoded by aminoglycoside 3'-phosphotransferase gene <i>aph(3')-II</i>]	<i>cat</i> sequence present <i>aph(3')-II</i> sequence present	<i>cat</i> sequence present <i>aph(3')-II</i> sequence present
Concentration by Qubit Fluorometer®	≥ 2 µg/mL	0.46 µg in 50 µL/vial (9.3 µg/mL)
Amount per Vial	Report results	0.46 µg/vial
OD₂₆₀/OD₂₈₀ Ratio (pre-vial)	1.7 to 2.1	1.98
Effective Bacterial Transformation Invitrogen™ MAX Efficiency™ Stbl2™ <i>E. coli</i>	≥ 50 colonies/ng	119 colonies/ng

¹The sequence was assembled pre-vial using the depositor's predicted sequence as the reference sequence. The complete plasmid sequence and map are provided on the BEI Resources webpage.

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05 JUN 2024

Technical Manager or designee, ATCC Federal Solutions

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