

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.^{1,2,3} 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. The deposited glycerol stock was grown in Luria-Bertani broth with chloramphenicol (12.5 µg per mL) for 1 day at 37°C in an aerobic atmosphere. The plasmid was extracted using a Plasmid *Plus* Maxi Kit (QIAGEN® 12963) and vialled in EB buffer (10 mM Tris-HCl, pH 8.5).

Lot: 70042538

Manufacturing Date: 01MAR2021

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 36000 base pairs	36024 base pairs ¹
Genotypic Analysis Sequencing of SARS-CoV-2 replicon (~ 28200 base pairs)	≥ 99% sequence identity to depositor sequence ≥ 99% sequence identity to SARS-CoV-2, Wuhan-Hu-1 (GenBank: NC_045512.2) Luc2-eGFP fusion sequence present poly A/HDV ribozyme sequence present	99.9% sequence identity to depositor sequence ² 100% sequence identity to SARS-CoV-2, Wuhan-Hu-1 (GenBank: NC_045512.2) ³ Luc2-eGFP fusion sequence present poly A/HDV ribozyme 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 PicoGreen® Measurement	≥ 2 µg per mL	0.1 µg in 20 µL per vial (5 µg per mL)
Amount per Vial	Report results	0.1 µg per vial
OD₂₆₀/OD₂₈₀ Ratio	1.7 to 2.1	1.9
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 <i>E. coli</i>	≥ 50 colonies per ng	64 colonies per 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.

²The sequence shows a two base pair insertion and two single nucleotide polymorphisms (snps) compared to the depositor sequence. These mutations are within the vector backbone and not expected to affect the function of NR-54972.

³Percentage alignment is determined without the S, M and E genes, which were removed from this construct.

/Heather Couch/

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25 MAR 2021

Program Manager or designee, ATCC Federal Solutions

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