

**Vector pMCSG53 Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Non-Structural Protein 16 Gene**

**Catalog No. NR-52427**

This reagent is the tangible property of the U.S. Government.

**Product Description:**

The non-structural protein 16 (nsp16) gene from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: [MN908947](#)) was codon optimized and cloned into the pMCSG53 plasmid. pMCSG53 is an *Escherichia coli* (*E. coli*) expression vector that contains an N-terminal hexa-histidine tag, followed by a tobacco etch virus (TEV) protease recognition site prior to the insert coding sequence, resulting in the expression of a cleavable histidine-tagged protein. It also contains tRNA genes covering rare codons for Arg (AGG/AGA) and Ile (AUA) to improve expression in *E. coli*. The beta-lactamase gene, *bla*, provides transformant selection through ampicillin resistance in *E. coli*. The deposited plasmid was transformed into One Shot™ TOP10 *Escherichia coli* (Invitrogen™ C404003), grown in Luria-Bertani broth with ampicillin (50 µg per mL) for 1 day at 37°C in an aerobic atmosphere, 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: 70035123**

**Manufacturing Date: 06MAY2020**

TEST	SPECIFICATIONS	RESULTS
<b>Next-Generation DNA Sequencing</b>	~ 5710 base pairs	5705 base pairs <sup>1</sup>
<b>Genotypic Analysis</b> Sequencing of nsp16 insert (900 base pairs) N-terminal His <sub>6</sub> tag N-terminal TEV protease site	100% sequence identity to depositor's sequence His <sub>6</sub> tag sequence confirmed TEV protease site sequence confirmed	100% sequence identity to depositor's sequence <sup>2</sup> His <sub>6</sub> tag sequence confirmed TEV protease site sequence confirmed
<b>Antibiotic Resistance</b> Ampicillin (encoded by beta-lactamase gene <i>bla</i> ) <sup>3</sup>	<i>bla</i> sequence present	<i>bla</i> sequence present
<b>Concentration by PicoGreen® Measurement</b>	≥ 2 µg/mL	0.2 µg in 20 µL per vial (7 µg/mL)
<b>Amount per Vial</b>	Report results	0.2 µg per vial
<b>OD<sub>260</sub>/OD<sub>280</sub> Ratio (pre-vial)</b>	1.7 to 2.1	1.9
<b>Effective Bacterial Transformation</b> Invitrogen™ One Shot™ TOP10 <i>Escherichia coli</i>	≥ 50 colonies per ng	366 colonies per ng

<sup>1</sup>The 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.

<sup>2</sup>The NR-52427 insert was codon optimized, but otherwise is consistent with the SARS-CoV-2, Wuhan-Hu-1 nsp16 protein (GenPept: YP\_009725311.1).

<sup>3</sup>The antibiotic ampicillin degrades quickly during growth. Bacterial stationary phase should be minimized during plasmid expansion to avoid plasmid loss and increased antibiotic concentrations may be necessary.

/Heather Couch/

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28 MAY 2020

Program Manager or designee, ATCC Federal Solutions

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