

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

**Catalog No. NR-52899**

**Product Description:**

The non-structural protein 1 (nsp1) 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 arginine (AGG/AGA) and isoleucine (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 *E. 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: 70036141**

**Manufacturing Date: 21MAY2020**

TEST	SPECIFICATIONS	RESULTS
<b>Next-Generation DNA Sequencing</b>	~ 5330 base pairs	5328 base pairs <sup>1</sup>
<b>Genotypic Analysis</b> Sequencing of nsp1 gene (~ 540 base pairs) Sequencing of pMCSG53 vector (~ 4790 base pairs)	≥ 99% sequence identity to depositor's sequence N-terminal hexa-histidine tag confirmed N-terminal TEV protease cleavage site confirmed	100% sequence identity to depositor's sequence <sup>2</sup> N-terminal hexa-histidine tag confirmed N-terminal TEV protease cleavage site 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 Qubit™ Measurement</b>	≥ 2 µg/mL	0.2 µg in 20 µL per vial (12 µg/mL)
<b>Amount per Vial</b>	Report results	0.2 µg per vial
<b>OD<sub>260</sub>/OD<sub>280</sub> Ratio</b>	1.7 to 2.1	1.9
<b>Effective Bacterial Transformation</b> Invitrogen™ One Shot™ TOP10 <i>E. coli</i>	≥ 50 colonies per ng	273 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. **Note:** NR-52899 contains a LacI mutation (C140Y); however, the mutation does not appear to affect plasmid function [see Meyer, S., et al. "Engineering Alternate Cooperative-Communications in the Lactose Repressor Protein Scaffold." *Protein Eng. Des. Sel.* 26 (2013): 433-443. PubMed: 23587523.].

<sup>2</sup>The NR-52899 insert was codon optimized but is 100% identical with the NSP1 within the ORF1ab polyprotein of SARS-CoV-2, Wuhan-Hu-1 (GenPept: QHD43415).

<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.

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Program Manager or designee, ATCC Federal Solutions

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