SUPPORTING INFECTIOUS DISEASE RESEARCH

# 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: <u>MN908947</u>) 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<sup>TM</sup> TOP10 *Escherichia coli* (Invitrogen<sup>TM</sup> 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<sup>®</sup> 12963) and vialed in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

# Lot: 70035123

## Manufacturing Date: 06MAY2020

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 5710 base pairs	5705 base pairs <sup>1</sup>
Genotypic Analysis		
Sequencing of nsp16 insert (900 base pairs)	100% sequence identity to depositor's sequence	100% sequence identity to depositor's sequence <sup>2</sup>
N-terminal His₀ tag N-terminal TEV protease site	His <sub>6</sub> tag sequence confirmed TEV protease site sequence confirmed	His <sub>6</sub> tag sequence confirmed TEV protease site sequence confirmed
Antibiotic Resistance		
Ampicillin (encoded by beta-lactamase gene bla)3	<i>bla</i> sequence present	bla sequence present
Concentration by PicoGreen <sup>®</sup> Measurement	≥ 2 µg/mL	0.2 μg in 20 μL per vial (7 μg/mL)
Amount per Vial	Report results	0.2 μg per vial
OD <sub>260</sub> /OD <sub>280</sub> Ratio (pre-vial)	1.7 to 2.1	1.9
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 Escherichia coli	≥ 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).

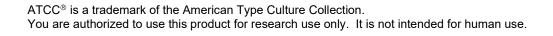
<sup>3</sup>The antibiotic ampicillin degrades quickly during growth. Bacterial stationary phase should be minimized during plasmid expansion to avoid plasmid loss and increased antibiotic concentrations may be necessary.

# /Heather Couch/

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28 MAY 2020

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