

Vector pMCSG53 Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Nucleocapsid Protein RNA Binding Domain Gene

Catalog No. NR-52429

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Product Description:

The RNA binding domain (RBD) of the nucleocapsid (N) protein 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: 70035128

Manufacturing Date: 06MAY2020

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 5190 base pairs	5192 base pairs ¹
Genotypic Analysis Sequencing of N RBD insert (~ 390 base pairs) N-terminal His ₆ tag N-terminal TEV protease site	100% sequence identity to depositor's sequence His ₆ tag sequence confirmed TEV protease site sequence confirmed	100% sequence identity to depositor's sequence ² His ₆ tag sequence confirmed TEV protease site sequence confirmed
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene <i>bla</i>) ³	<i>bla</i> sequence present	<i>bla</i> sequence present
Concentration by PicoGreen® Measurement	≥ 2 µg/mL	0.2 µg in 20 µL per vial (12 µg/mL)
Amount per Vial	Report results	0.2 µg per vial
OD₂₆₀/OD₂₈₀ Ratio (pre-vial)	1.7 to 2.1	1.9
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 <i>Escherichia coli</i>	≥ 50 colonies per ng	62 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 NR-52429 insert was codon optimized, but otherwise is consistent with the SARS-CoV-2, Wuhan-Hu-1 N RBD protein (GenPept: YP_009724397.2).

³The 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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26 MAY 2020

Program Manager or designee, ATCC Federal Solutions

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