b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-52423

Vector pMCSG53 Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 SARS-CoV Unique Domain Gene

Catalog No. NR-52423

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

Product Description:

The SARS-CoV unique domain (SUD) 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 vialed in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Lot: 70035115 **Manufacturing Date: 06MAY2020**

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 5600 base pairs	5603 base pairs[1]
Genotypic Analysis Sequencing of SUD insert (~ 790 base pairs) Sequencing of pMCSG53 vector N-terminal His_6 tag N-terminal TEV protease site	100% sequence identity to depositor's sequence His_6 tag sequence present TEV protease site sequence present	100% sequence identity to depositor's sequence His_6 tag sequence present TEV protease site sequence present
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene *bla*)[2]	*bla* sequence present	*bla* sequence present
Concentration by PicoGreen® Measurement	≥ 2 µg/mL	0.3 µg in 30 µL per vial (9 µg/mL)
Amount per Vial	Report results	0.3 µg per vial
OD_{260}/OD_{280} Ratio (pre-vial)	1.7 to 2.1	2.0
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 *Escherichia coli*	≥ 50 colonies per ng	242 colonies per ng

[1]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.
[2]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/
Heather Couch 18 MAY 2020
Program Manager or designee, ATCC Federal Solutions

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