b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-52428

Vector pMCSG53 Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike Glycoprotein N-Terminal Domain

Catalog No. NR-52428

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

Product Description:

The vector for the N-terminal domain (NTD) of the spike (S) glycoprotein gene from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: MN908947) was designed by subcloning the codon-optimized S protein NTD (amino acids 26 to 305) into the pMCSG53 *Escherichia coli (E. coli)* expression vector. pMCSG53 is a ligation-independent cloning (LIC) vector containing an N-terminal hexa-histidine tag and tobacco etch virus (TEV) protease recognition site prior to the NTD. In addition, the vector includes tRNA genes covering rare codons for arginine (AGG/AGA) and isoleucine (AUA) to improve expression in the host, *E. coli*. NR-52428 contains the beta-lactamase gene, *bla*, to provide 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 vialed in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Lot: 70035126 Manufacturing Date: 20APR2020

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 5650 base pairs	5651 base pairs[1]
Genotypic Analysis Sequencing of Spike NTD insert (~ 850 base pairs) Sequencing of pMCSD53 vector	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 *bla*)[3]	*bla* sequence present	*bla* sequence present
Concentration by PicoGreen® Measurement	≥ 2 µg/mL	0.04 µg in 20 µL per vial (2.2 µg/mL)
Amount per Vial	Report results	0.04 µg per vial
OD_{260}/OD_{280} Ratio	1.7 to 2.1	1.9
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 *E. coli*	≥ 50 colonies per ng	88 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 NR-52428 insert was codon optimized but is consistent with the SARS-CoV-2, Wuhan-Hu-1 S protein (GenPept: QHD43416).
[3]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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Program Manager or designee, ATCC Federal Solutions

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