b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-52430

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

Catalog No. NR-52430

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

Product Description:

The vector for the receptor binding domain (RBD) 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 RBD (amino acids 319 to 542) 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 RBD. 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-52430 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: 70035130 **Manufacturing Date: 20APR2020**

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 5500 base pairs	5483 base pairs[1]
Genotypic Analysis Sequencing of Spike RBD insert (~ 680 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	0.2 µg in 20 µL per vial (11.6 µg/mL)
Amount per Vial	Report results	0.2 µg per vial
OD_{260}/OD_{280} Ratio (pre-vial)	1.7 to 2.1	2.0
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 *E. coli*	 ≥ 50 colonies per ng	 67 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-52430 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/
Heather Couch 01 MAY 2020
Program Manager or designee, ATCC Federal Solutions

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Support Provided by NIAID

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