b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-52421

Vector pCMV Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike Glycoprotein Ectodomain

Catalog No. NR-52421

Product Description:

The vector for the spike (S) glycoprotein gene from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: MN908947) was designed by codon optimizing the S glycoprotein ectodomain (residues 14 to 1211) for mammalian expression, fused to an N-terminal mu-phosphatase signal sequence and C-terminal trimerizing foldon domain and octa-histidine tag, and subcloned into the pCMV mammalian expression vector. The recombinant protein is stabilized by substitution at the furin S1/S2 cleavage site (RRAR→SGAG; residues 682 to 685) and KV→PP mutations (residues 983 and 984). NR-52421 contains the beta-lactamase gene, TEM-116, to provide transformant selection through ampicillin resistance in *Escherichia coli (E. coli),* and a neomycin (G418) selectable marker for mammalian expression. The deposited plasmid was transformed into One Shot™ TOP10 *E. coli* (Invitrogen™ C404010), 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: 70035175 **Manufacturing Date: 20APR2020**

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	Report results	9297 base pairs[1]
Genotypic Analysis Sequencing of S glycoprotein insert (~ 3850 base pairs)	Report results N-terminal mu-phosphatase sequence confirmed C-terminal trimerizing foldon domain confirmed C-terminal His_8 confirmed	100% sequence identity to depositor's sequence[2] N-terminal mu-phosphatase sequence confirmed[3] C-terminal trimerizing foldon domain confirmed C-terminal His_8 confirmed
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene TEM-116)[4] Neomycin [encoded by aminoglycoside 3' phosphotransferase gene aph(3')-II]	TEM-116 sequence present aph(3')-II sequence present	TEM-116 sequence present aph(3')-II sequence present
Concentration by PicoGreen® Measurement	Report results	0.3 µg in 20 µL per vial (16 µg/mL)
Amount per Vial	Report results	0.3 µg per vial
OD_{260}/OD_{280} Ratio (pre-vial)	1.7 to 2.1	1.9
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 *E. coli*	≥ 50 colonies per ng	78 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-52421 insert was codon optimized for mammalian expression but has a 99% amino acid identity with the SARS-CoV-2, Wuhan-Hu-1 S protein residues 14 to 1211 (GenPept: QHD43416), with the stabilizing mutations confirmed.

[3]The mu-phosphatase signal sequence amino acid sequence is MGILPSPGMPALLSLVSLLSVLLMGCVAETGT.

[4]The antibiotic ampicillin degrades quickly during growth. Bacterial stationary phase should be minimized during plasmid replication to avoid plasmid loss and increased antibiotic concentrations may be necessary.

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/Heather Couch/
Heather Couch 24 AUG 2020
Program Manager or designee, ATCC Federal Solutions

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

Support Provided by NIAID

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