b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-53765

Vector pHDM Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike Glycoprotein Gene, D614G Mutant with C-Terminal Deletion

Catalog No. NR-53765

Product Description:

Note: The vial label indicates this product has a 21 base pair deletion but is a 21 amino acid deletion. 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 optimization of the S glycoprotein sequence (residues 1 to 1252) with a D614G mutation and deletion of the C-terminal 21 amino acids, and subcloned into the pHDM vector under the CMV promoter. NR-53765 contains the beta-lactamase gene, *bla*, to provide transformant selection through ampicillin resistance in *Escherichia coli (E. coli)*. The deposited plasmid was transformed into One Shot™ TOP10 *E. coli* (Invitrogen™ C404003), grown in Luria-Bertani broth with ampicillin (100 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: 70039033 **Manufacturing Date: 12AUG2020**

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 8310 base pairs	8316 base pairs[1]
Genotypic Analysis Sequencing of S glycoprotein insert (~ 3760 base pairs)	≥ 99% sequence identity to depositor's sequence	100% sequence identity to depositor's sequence[2]
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene *bla*)[3]	*bla* sequence present	*bla* sequence present
Concentration by PicoGreen® Measurement	≥ 2 µg/mL	0.6 µg in 30 µL per vial (19 µg/mL)
Amount per Vial	Report results	0.6 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	156 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]NR-53765 insert was codon optimized for mammalian expression with the D614G mutation, but otherwise is consistent with the SARS-CoV-2, Wuhan-Hu-1 S protein (GenPept: YP_009724390; residues 1-1252).
[3]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.

/Heather Couch/
Heather Couch 22 JAN 2021
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 by ATCC® and the contributor 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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