b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-53501

Vector pET-28a(+) Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Non-Structural Protein 9 Gene

Catalog No. NR-53501

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

Product Description:

The non-structural protein 9 (nsp9) gene from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: MN908947) was codon optimized, tagged with a tobacco etch virus (TEV) cleavable N-terminal hexa-histidine tag and cloned into the pET-28a(+) plasmid. The kanamycin resistance gene, *aph*, provides transformant selection through kanamycin 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 kanamycin (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: 70036466 **Manufacturing Date: 04JUN2020**

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 5650 base pairs	5646 base pairs[1]
Genotypic Analysis Sequencing of Nsp9 insert (~ 340 base pairs)	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 Kanamycin (encoded by *aph*)	*aph* sequence present	*aph* sequence present
Concentration by Qubit™ Measurement	≥ 2 µg/mL	0.3 µg in 20 µL per vial (14 µg/mL)
Amount per Vial	Report results	0.3 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	256 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-53501 insert was codon optimized but 100% identical with the SARS-CoV-2, Wuhan-Hu-1 NSP9 protein (GenPept: QHD43415).

/Heather Couch/
Heather Couch 27 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 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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