

Vector pET-28a(+) Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Non-Structural Protein 14 Gene

Catalog No. NR-53505

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

Product Description:

The non-structural protein 14 (nsp14) gene from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: [MN908947](#)) was codon optimized, tagged with a C-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 vialied in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Lot: 70036470

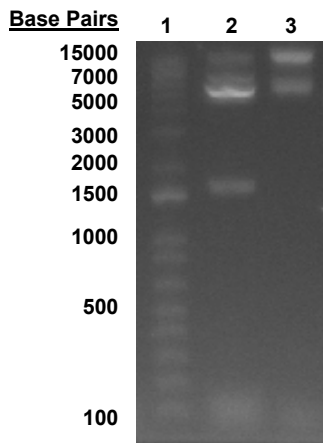
Manufacturing Date: 04JUN2020

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 6830 base pairs	6826 base pairs ¹
Genotypic Analysis Sequencing of nsp14 insert (~ 1580 base pairs)	100% sequence identity to depositor's sequence ² His ₆ tag sequence confirmed	100% sequence identity to depositor's sequence ² His ₆ tag sequence confirmed
Antibiotic Resistance Kanamycin (encoded by <i>aph</i>)	<i>aph</i> sequence present	<i>aph</i> sequence present
Agarose Gel Electrophoresis Digestion with <i>Xho</i> I and <i>Xba</i> I (pre-vial)	~ 5 kb and ~ 1.6 kb	~ 5 kb and ~ 1.6 kb (Figure 1)
Concentration by Qubit™ Measurement	≥ 2 µg/mL	0.3 µg in 20 µL per vial (15 µg/mL)
Amount per Vial	Report results	0.3 µg per vial
OD₂₆₀/OD₂₈₀ Ratio	1.7 to 2.1	1.9
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 <i>Escherichia coli</i>	≥ 50 colonies per ng	135 colonies per ng

¹The 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.

²The NR-53505 insert was codon optimized but otherwise is 100% identical with the SARS-CoV-2, Wuhan-Hu-1 NSP14 protein (GenPept: QHD43415).

Figure 1: Agarose Gel of Undigested and Restriction Enzyme Digested NR-53505



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
 Lane 2: NR-53505 digested
 Lane 3: NR-53505 undigested

/Heather Couch/
 Heather Couch

25 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.

ATCC® is a trademark of the American Type Culture Collection.
 You are authorized to use this product for research use only. It is not intended for human use.

