

**Vector pET-28a(+) Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Non-Structural Protein 4 Gene**

**Catalog No. NR-53497**

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

**Product Description:**

The non-structural protein 4 (nsp4) 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 vialied in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

**Lot: 70036462**

**Manufacturing Date: 03JUN2020**

TEST	SPECIFICATIONS	RESULTS
<b>Next-Generation DNA Sequencing</b>	~ 6810 base pairs	6807 base pairs <sup>1</sup>
<b>Genotypic Analysis</b> Sequencing of nsp4 insert (1500 base pairs)	100% sequence identity to depositor's sequence His <sub>6</sub> tag sequence confirmed TEV protease site sequence confirmed	100% sequence identity to depositor's sequence <sup>2</sup> His <sub>6</sub> tag sequence confirmed TEV protease site sequence confirmed
<b>Antibiotic Resistance</b> Kanamycin (encoded by <i>aph</i> )	<i>aph</i> sequence present	<i>aph</i> sequence present
<b>Concentration by Qubit™ Measurement</b>	≥ 2 µg/mL	0.2 µg in 20 µL per vial (9 µg/mL)
<b>Amount per Vial</b>	Report results	0.2 µg per vial
<b>OD<sub>260</sub>/OD<sub>280</sub> Ratio</b>	1.7 to 2.1	1.9
<b>Effective Bacterial Transformation</b> Invitrogen™ One Shot™ TOP10 <i>E. coli</i>	≥ 50 colonies per ng	198 colonies per ng

<sup>1</sup>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.

<sup>2</sup>The NR-53497 insert was codon optimized but otherwise is 100% identical with the SARS-CoV-2, Wuhan-Hu-1 NSP4 protein (GenPept: QHD43415).

/Heather Couch/

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04 SEP 2020

Program Manager or designee, ATCC Federal Solutions

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