

**Vector pαH Containing the Human Coronavirus, NL63 Recombinant Spike Ectodomain Gene**

**Catalog No. NR-54976**

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

**Product Description:**

NR-54976 is an expression vector containing the human coronavirus, NL63 recombinant spike ectodomain gene insert (codon optimized) encoding S1 ectodomain residues 1-1291 (GenPept: [Q6Q1S2.1](#)) linked to C-terminal T4 fibrin trimerization domain (foldon), an HRV3C cleavage site, octa His-tag and 2X Strep-tag® II. Recombinant S ectodomain trimer is stabilized in the prefusion conformation by two proline substitutions (S1052P and I1053P)<sup>1,2</sup> NR-54976 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 *Escherichia coli* (Invitrogen™ C404003), grown in Terrific 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 vialled in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

**Lot: 70043623**

**Manufacturing Date: 18MAY2021**

TEST	SPECIFICATIONS	RESULTS
<b>Next-Generation DNA Sequencing (pre-vial)</b>	~ 8080 base pairs	8071 base pairs <sup>1</sup>
<b>Genotypic Analysis</b> Sequencing of S glycoprotein insert (~ 4110 base pairs)	≥ 99% sequence identity to depositor's sequence C-terminal T4 foldon trimerization domain confirmed C-terminal HRV3C protease cleavage site confirmed C-terminal octa-histidine tag confirmed C-terminal 2X Strep-tag® II confirmed	99.9% sequence identity to depositor's sequence <sup>2</sup> C-terminal T4 foldon trimerization domain confirmed C-terminal HRV3C protease cleavage site confirmed C-terminal octa-histidine tag confirmed C-terminal 2X Strep-tag® II confirmed
<b>Antibiotic Resistance</b> Ampicillin (encoded by beta-lactamase gene <i>bla</i> )	<i>bla</i> sequence present	<i>bla</i> sequence present
<b>Concentration by PicoGreen® Measurement</b>	≥ 2 µg/mL	0.6 µg in 30 µL/vial (19 µg/mL)
<b>Amount per Vial</b>	Report results	0.6 µg/vial
<b>OD<sub>260</sub>/OD<sub>280</sub> Ratio</b>	1.7 to 2.1	2.0
<b>Effective Bacterial Transformation</b> Invitrogen™ One Shot™ TOP10 <i>E. coli</i>	≥ 50 colonies/ng	59 colonies/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-54976 insert was codon optimized for mammalian expression but amino acid identity is consistent with human coronavirus, NL63 S glycoprotein (GenPept: AFV53148.1) other than the stabilization mutations and four novel SNP mutations: c2668a, a2670t, t2673c, and a2675c result in the following effects: -- (silent mutation), N486Y, F487S, and T488P, respectively. The effect of these mutations on the function of S is not known.

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