

**Modified p<sub>α</sub>H Vector Containing the Human Coronavirus, OC43 Spike Glycoprotein**

**Catalog No. NR-54979**

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

**Product Description:**

The vector for the spike (S) glycoprotein gene from human coronavirus, OC43 (GenBank: [KF572804](#)) was designed by codon optimizing the full-length S sequence (residues 1 to 1287) for mammalian expression and subcloning into the p<sub>α</sub>H mammalian expression vector, which was modified by subcloning a T4 foldon trimerization domain, HRV3C protease cleavage site, and the octa-histidine and 2X *Strep-tag*<sup>®</sup> II tags downstream of the open reading frame. The recombinant protein is stabilized by AL→PP mutations (residues 1079 and 1080). NR-54979 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 vialied in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

**Lot: 70043626**

**Manufacturing Date: 18MAY2021**

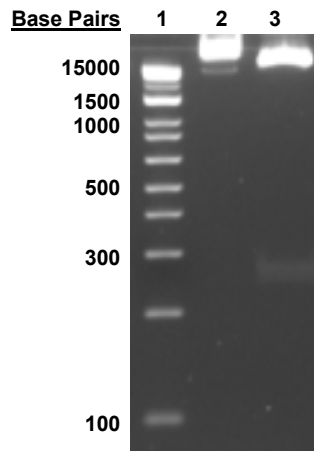
TEST	SPECIFICATIONS	RESULTS
<b>Next-Generation DNA Sequencing (pre-vial)</b>	Report results	8,064 base pairs <sup>1</sup>
<b>Genotypic Analysis</b> Sequencing of S glycoprotein insert (~ 4,000 base pairs)	≥ 99% sequence identity to depositor's sequence C-terminal HRV3C protease cleavage site confirmed C-terminal T4 foldon trimerization domain confirmed C-terminal octa-histidine tag confirmed C-terminal 2X <i>Strep-tag</i> <sup>®</sup> II confirmed	100% sequence identity to depositor's sequence <sup>2</sup> C-terminal HRV3C protease cleavage site confirmed C-terminal T4 foldon trimerization domain confirmed C-terminal octa-histidine tag confirmed C-terminal 2X <i>Strep-tag</i> <sup>®</sup> II confirmed
<b>Antibiotic Resistance</b> Ampicillin (encoded by beta-lactamase gene <i>bla</i> ) <sup>3</sup>	<i>bla</i> sequence present	<i>bla</i> sequence present
<b>Agarose Gel Electrophoresis (pre-vial)</b> Digestion with <i>Bam</i> HI and <i>Xho</i> I	~ 7 kb and 300 bp	~ 7 kb and 300 bp (Figure 1)
<b>Concentration by PicoGreen<sup>®</sup> Measurement</b>	≥ 2 µg per mL	0.7 µg in 30 µL per vial (23 µg per mL)
<b>Amount per Vial</b>	Report results	0.7 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	58 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-54979 insert was codon optimized for mammalian expression but has 100% amino acid identity with human coronavirus, OC43 S glycoprotein (GenPept: AIL49484.1) other than the stabilization mutations.

<sup>3</sup>The antibiotic ampicillin degrades quickly during growth. Bacterial stationary phase should be minimized during plasmid expansion to avoid plasmid loss and increased antibiotic concentrations may be necessary.

Figure 1: Agarose Gel of Undigested and Restriction Enzyme Digested NR-54979



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder  
 Lane 2: NR-54979 undigested  
 Lane 3: NR-54979 digested

/Heather Couch/  
 Heather Couch

26 MAY 2022

Program Manager or designee, ATCC Federal Solutions

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