

**Modified p*α*H Vector Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike Glycoprotein**

**Catalog No. NR-52564**

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

**Product Description:**

Note: The label on the vial is incorrect; the expressed protein is untagged. NR-52564 expresses the full-length, unmodified S glycoprotein, and is intended for producing pseudotyped particles/pseudovirions. NR-52564 is not intended for recombinant protein expression. The vector for the spike (S) glycoprotein gene from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: [MN908947](#)) was designed by codon optimizing the full-length S sequence (residues 1 to 1273) for mammalian expression and subcloning into the p*α*H mammalian expression vector, which was modified by subcloning a T4 foldon trimerization domain, HRV3C protease cleavage site, and the tags Twin-Strep-tag® (TST) and octa-histidine downstream of the open reading frame. However, NR-52564 expresses the full-length S protein without any additional tags or modifications. NR-52564 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 *E. coli* (Invitrogen™ C404010), 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: 70035521**

**Manufacturing Date: 01MAY2020**

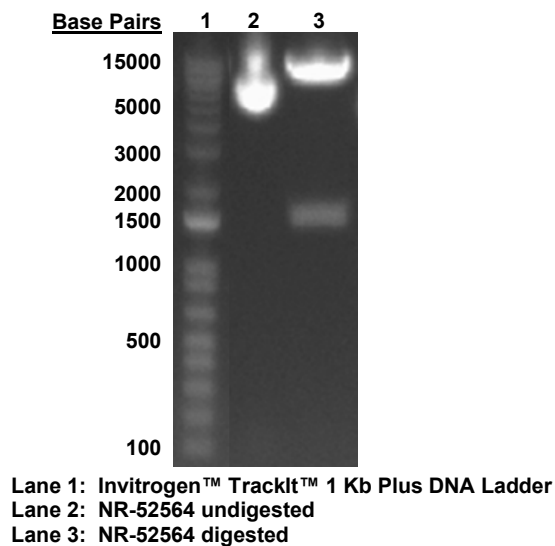
TEST	SPECIFICATIONS	RESULTS
<b>Next-Generation DNA Sequencing</b>	~ 8570 base pairs	8573 base pairs <sup>1</sup>
<b>Genotypic Analysis</b> Sequencing of S glycoprotein insert (~ 3820 base pairs) Sequencing of modified p <i>α</i> H vector (~ 4750 base pairs)	≥ 99% sequence identity to depositor's sequence T4 foldon trimerization domain sequence confirmed HRV3C protease site sequence confirmed TST sequence confirmed His <sub>8</sub> tag sequence confirmed	99.9% sequence identity to depositor's sequence <sup>2</sup> T4 foldon trimerization domain sequence confirmed HRV3C protease site sequence confirmed TST sequence confirmed His <sub>8</sub> tag sequence 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 (post-vial)</b> Digestion with <i>SapI</i>	~ 7 kb and ~ 1.4 kb	~ 7 kb and ~ 1.4 kb (Figure 1)
<b>Concentration by PicoGreen® Measurement</b>	≥ 2 µg/mL	0.7 µg in 30 µL per vial (23 µg/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	92 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-52564 insert was codon optimized for mammalian expression but has a 100% amino acid identity with the SARS-CoV-2, Wuhan-Hu-1 S protein (GenPept: QHD43416).

<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-52564**



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Program Manager or designee, ATCC Federal Solutions

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