

Modified pαH Vector Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike Glycoprotein Ectodomain

Catalog No. NR-52563

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Product Description:

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 S sequence ectodomain (residues 1 to 1208) for mammalian expression and subcloning into the pαH mammalian expression vector. The recombinant protein is stabilized by substitution at the furin S1/S2 cleavage site (RRAR→GSAS; residues 682 to 685) and KV→PP mutations (residues 986 and 987). The pαH vector was modified by subcloning an SV40 promoter upstream of the S gene insert, as well as subcloning a T4 foldon trimerization domain, HRV3C protease cleavage site, and the tags Twin-Strep-tag® (TST) and octa-histidine downstream of the S gene. NR-52463 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), grown in Luria-Bertani broth with ampicillin (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: 70035520

Manufacturing Date: 01MAY2020

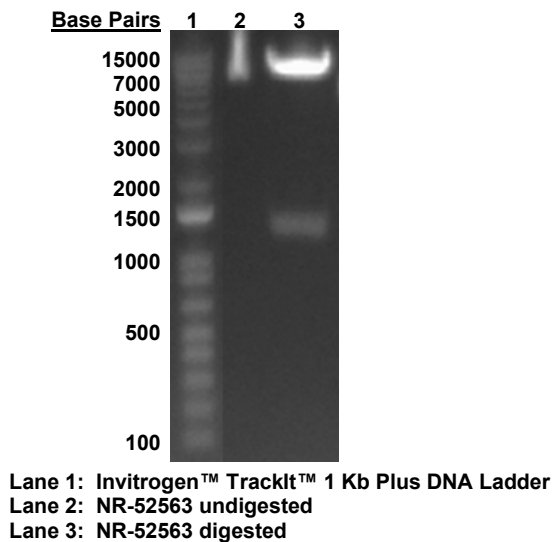
TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 8370 base pairs	8375 base pairs ¹
Genotypic Analysis Sequencing of S glycoprotein insert (~ 3620 base pairs) Sequencing of modified pαH vector (~ 4750 base pairs)	100% sequence identity to depositor's sequence T4 foldon trimerization domain sequence confirmed HRV3C protease site sequence confirmed TST sequence confirmed His ₈ tag sequence confirmed	100% sequence identity to depositor's sequence ² T4 foldon trimerization domain sequence confirmed HRV3C protease site sequence confirmed TST sequence confirmed His ₈ tag sequence confirmed
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene <i>bla</i>) ³	<i>bla</i> sequence present	<i>bla</i> sequence present
Agarose Gel Electrophoresis Digestion with <i>SapI</i> (pre-vial)	~ 7 kb and ~ 1.2 kb	~ 7 kb and ~ 1.2 kb (Figure 1)
Concentration by PicoGreen® Measurement	≥ 2 µg/mL	0.5 µg in 20 µL per vial (27 µg/mL)
Amount per Vial	Report results	0.5 µg per vial
OD₂₆₀/OD₂₈₀ Ratio	1.7 to 2.1	1.9
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 <i>E. coli</i>	≥ 50 colonies per ng	182 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-52563 insert was codon optimized for mammalian expression with mutations for stability and solubility, but otherwise is consistent with the SARS-CoV-2, Wuhan-Hu-1 S protein (GenPept: QHD43416; residues 1 to 1208).

³The 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-52563



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