

Modified p α H Vector Containing the Middle East Respiratory Syndrome Coronavirus Spike Glycoprotein

Catalog No. NR-54980

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Product Description:

The vector for the spike (S) glycoprotein gene from middle east respiratory syndrome coronavirus (MERS-CoV), England 1 (GenBank: [NC_038294](#)) was designed by codon optimizing the full-length S sequence (residues 1 to 1291) for mammalian expression and subcloning into the p α H mammalian expression vector, which was modified by subcloning a T4 foldon trimerization domain, a HRV3C protease cleavage site and the octa-histidine and 2X Strep-tag[®] II tags downstream of the open reading frame. The recombinant protein is stabilized by substitution at the furin S1/S2 cleavage site (RVSR \rightarrow ASVG; residues 748 to 751) and VL \rightarrow PP mutations (residues 1060 and 1061). NR-54980 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 μ g per mL) for 1 day at 37 $^{\circ}$ 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: 70043627

Manufacturing Date: 18MAY2021

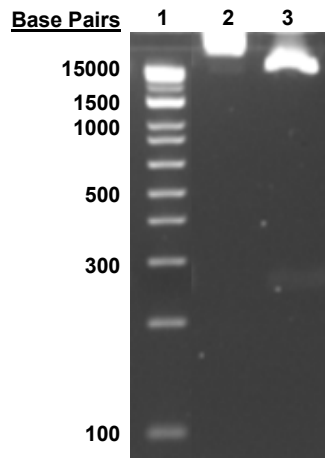
TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing (pre-vial)	Report results	8070 base pairs ¹
Genotypic Analysis Sequencing of S glycoprotein insert (~ 4000 base pairs)	\geq 99% sequence identity to depositor's sequence C-terminal HRV3C protease cleavage site confirmed C-terminal T4 foldon trimerization domain confirmed C-terminal octa-histidine tag confirmed C-terminal 2X Strep-tag [®] II confirmed	100% sequence identity to depositor's sequence ² C-terminal HRV3C protease cleavage site confirmed C-terminal T4 foldon trimerization domain confirmed C-terminal octa-histidine tag confirmed C-terminal 2X Strep-tag [®] II 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 (pre-vial) Digestion with <i>Bam</i> HI and <i>Xho</i> I	~ 7 kb and 300 bp	~ 7 kb and 300 bp (Figure 1)
Concentration by PicoGreen[®] Measurement	\geq 2 μ g per mL	0.1 μ g in 20 μ L per vial (6 μ g per mL)
Amount per Vial	Report results	0.1 μ g per vial
OD₂₆₀/OD₂₈₀ Ratio	1.7 to 2.1	1.8
Effective Bacterial Transformation Invitrogen [™] One Shot [™] TOP10 <i>E. coli</i>	\geq 50 colonies per ng	214 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-54980 insert was codon optimized for mammalian expression but has 100% amino acid identity with MERS-CoV S glycoprotein (GenPept: YP_007188579.1) other than the stabilization mutations.

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-54980



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
 Lane 2: NR-54980 undigested
 Lane 3: NR-54980 digested

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