Certificate of Analysis for NR-53587

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

Catalog No. NR-53587

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

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) as well as the additional proline substitutions that create the HexaPro variant (F817P, A892P, A899P and A942P). 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. The ampicillin resistance gene, *bla*, provides transformant selection through ampicillin resistance in *Escherichia coli (E. coli)*. The deposited plasmid was transformed into One Shot™ TOP10 *E. coli* (Invitrogen™ C404003), grown in Luria-Bertani 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 vialed in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Lot: 70037523 **Manufacturing Date: 14JUL2020**

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 8370 base pairs	8376 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 *bla*)[3]	*bla* sequence present	*bla* sequence present
Agarose Gel Electrophoresis Digestion with *Xho*I and *Kpn*I (pre-vial)	~ 5 kb and ~ 4 kb	~ 5 kb and ~ 4 kb (Figure 1)
Concentration by Qubit™ Measurement	≥ 2 µg/mL	0.8 µg in 50 µL per vial (16 µg/mL)
Amount per Vial	Report results	0.8 µg per vial
OD_{260}/OD_{280} Ratio	1.7 to 2.1	1.8
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 *E. coli*	≥ 50 colonies per ng	130 colonies per ng

[1]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.
[2]The NR-53587 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).
[3]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.

BEI Resources
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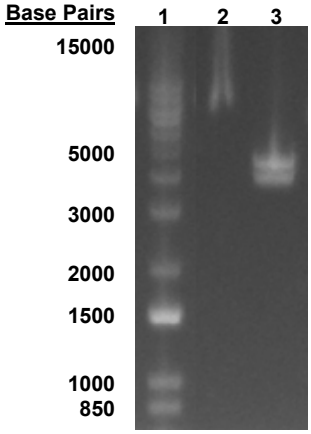
Figure 1: Agarose Gel of Undigested and Restriction Enzyme Digested NR-53587



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
Lane 2: NR-53587 undigested
Lane 3: NR-53587 digested

/Heather Couch/
Heather Couch 14 SEP 2020
Program Manager or designee, ATCC Federal Solutions

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Support Provided by NIAID

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