Certificate of Analysis for NR-54975

Vector pαH Containing the SARS Coronavirus, Recombinant Spike Ectodomain Gene

Catalog No. NR-54975

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

Product Description:

NR-54975 is an expression vector containing the SARS coronavirus, recombinant spike ectodomain gene insert (codon optimized) encoding S1 ectodomain residues 1-1190 (GenPept: AAP41037.1) linked to C-terminal T4 fibritin trimerization domain (foldon), an HRV3C cleavage site, octa His-tag and Strep-tag® II. Recombinant S ectodomain trimer is stabilized in the prefusion conformation by two proline substitutions (K968P and V969P)[1,2] NR-54975 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 vialed in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Lot: 70043622 **Manufacturing Date: 18MAY2021**

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing (pre-vial)	~ 7710 base pairs	7709 base pairs[1]
Genotypic Analysis Sequencing of S glycoprotein insert (~ 3600 base pairs)	≥ 99% sequence identity to depositor's sequence C-terminal T4 foldon trimerization domain confirmed C-terminal HRV3C protease cleavage site confirmed C-terminal octa-histidine tag confirmed C-terminal Strep-tag® II confirmed	100% sequence identity to depositor's sequence[2] C-terminal T4 foldon trimerization domain confirmed C-terminal HRV3C protease cleavage site confirmed C-terminal octa-histidine tag confirmed C-terminal Strep-tag® II confirmed
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene *bla*)	*bla* sequence present	*bla* sequence present
Concentration by PicoGreen® Measurement	≥ 2 µg/mL	0.6 µg in 30 µL/vial (20 µg/mL)
Amount per Vial	Report results	0.6 µg/vial
OD_{260}/OD_{280} Ratio	1.7 to 2.1	1.9
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 *E. coli*	≥ 50 colonies/ng	56 colonies/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-54975 insert was codon optimized for mammalian expression, amino acid identity is consistent with SARS-CoV S glycoprotein (GenPept: AAP41037.1) other than the stabilization mutations.

BEI Resources
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/Sonia Bjorum Brower/
Sonia Bjorum Brower 01 JUN 2023
Technical Manager or designee, ATCC Federal Solutions

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Support Provided by NIAID

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