

Vector pαH Containing the Human Coronavirus, 229E Recombinant Spike Ectodomain Gene

Catalog No. NR-54977

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Product Description:

NR-54977 is an expression vector containing the human coronavirus, 229E recombinant spike ectodomain gene insert (codon optimized) encoding S1 ectodomain residues 1-1108 (GenPept: [AOG74783.1](#)) linked to C-terminal T4 fibrin trimerization domain (foldon), an HRV3C cleavage site, octa His-tag and 2X Strep-tag® II. Recombinant S ectodomain trimer is stabilized in the prefusion conformation by two proline substitutions (I869P and I870P)^{1,2} NR-54977 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 vialled in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Lot: 70043624

Manufacturing Date: 18MAY2021

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing (pre-vial)	~ 7530 base pairs	7522 base pairs ¹
Genotypic Analysis Sequencing of S glycoprotein insert (~ 3560 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 2X 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 2XStrep-tag® II confirmed
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene <i>bla</i>)	<i>bla</i> sequence present	<i>bla</i> sequence present
Concentration by PicoGreen® Measurement	≥ 2 µg/mL	0.4 µg in 20 µL/vial (20 µg/mL)
Amount per Vial	Report results	0.4 µg/vial
OD₂₆₀/OD₂₈₀ Ratio	1.7 to 2.1	2.0
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 <i>E. coli</i>	≥ 50 colonies/ng	51 colonies/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-54977 insert was codon optimized for mammalian expression but has 100% amino acid identity with human coronavirus, 229E S glycoprotein (GenPept: AOG74783.1) other than the stabilization mutations.

/Sonia Bjorum Brower/

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01 JUN 2023

Technical Manager or designee, ATCC Federal Solutions

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