

Modified pH Vector Containing the Human Coronavirus, HKU1 Spike Glycoprotein

Catalog No. NR-54978

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

Product Description:

The vector for the spike (S) glycoprotein gene from human coronavirus, HKU1 was designed by codon optimizing the full-length S sequence (residues 1 to 1277; GenPept: [Q0ZME7](#)) for mammalian expression and subcloning into the pH mammalian expression vector, which was modified by subcloning a HRV3C protease cleavage site, T4 foldon trimerization domain, and the octa-histidine and Strep-tag® II tags downstream of the open reading frame. The recombinant protein is stabilized by substitution at the furin S1/S2 cleavage site (RRKRR→GGSGS; residues 752 to 756) and NL→PP mutations (residues 1067 and 1068). NR-54978 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™ C404010), 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: 70043625

Manufacturing Date: 18MAY2021

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing (pre-vial)	7974 base pairs	~ 7970 base pairs ¹
Genotypic Analysis Sequencing of S glycoprotein insert (~ 4000 base pairs)	≥ 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 <i>Strep-tag</i> ® 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 <i>Strep-tag</i> ® II confirmed
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene <i>bla</i>) ³	<i>bla</i> sequence present	<i>bla</i> sequence present
Concentration by PicoGreen® Measurement	≥ 2 µg per mL	0.5 µg in 30 µL per vial (18 µg per 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	74 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-54978 insert was codon optimized for mammalian expression but has 100% amino acid identity with human coronavirus, HKU1 S glycoprotein (GenPept: Q0ZME7) 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.

/Heather Couch/

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27 MAY 2022

Program Manager or designee, ATCC Federal Solutions

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