

Vector pcDNA3.1(-) Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike Glycoprotein Receptor Binding Domain (RBD)

Catalog No. NR-52422

Product Description:

The vector for the spike (S) glycoprotein receptor binding domain (RBD; also referred to as S^B) from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: [MN908947](#)) was designed by codon optimizing the spike RBD (amino acids 328 to 531) for mammalian expression, fused to an N-terminal mu-phosphatase signal sequence and C-terminal octa-histidine tag, and subcloned into the [pcDNA™3.1\(-\)](#) mammalian expression vector. NR-52422 contains the beta-lactamase gene, TEM-116, to provide transformant selection through ampicillin resistance in *Escherichia coli* (*E. coli*), and a neomycin (G418) selectable marker for mammalian expression. The deposited plasmid was transformed into One Shot™ TOP10 *E. coli* (Invitrogen™ C404010), grown in Luria-Bertani broth with ampicillin (50 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: 70035172

Manufacturing Date: 20APR2020

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	Report results	6115 base pairs ¹
Genotypic Analysis Sequencing of spike RBD insert (~ 730 base pairs)	Report results N-terminal mu-phosphatase sequence confirmed C-terminal His ₈ tag confirmed	100% sequence identity to depositor's sequence ² N-terminal mu-phosphatase sequence confirmed ³ C-terminal His ₈ tag confirmed
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene TEM-116) ⁴ Neomycin [encoded by aminoglycoside 3'-phosphotransferase gene aph(3')-II]	TEM-116 sequence present aph(3')-II sequence present	TEM-116 sequence present aph(3')-II sequence present
Concentration by PicoGreen® Measurement	Report results	0.4 µg in 20 µL per vial (21 µg/mL)
Amount per Vial	Report results	0.4 µg per vial
OD₂₆₀/OD₂₈₀ Ratio (pre-vial)	1.7 to 2.1	1.9
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 <i>E. coli</i>	≥ 50 colonies per ng	78 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-52422 insert was codon optimized for mammalian expression but has a 100% amino acid identity with the SARS-CoV-2, Wuhan-Hu-1 S protein residues 328 to 531 (GenPept: QHD43416).

³The translated mu-phosphatase signal sequence is MGILPSPGMPALLSLVLLSLLVMGCV AETGT.

⁴The antibiotic ampicillin degrades quickly during growth. Bacterial stationary phase should be minimized during plasmid replication to avoid plasmid loss and increased antibiotic concentrations may be necessary.

/Heather Couch/

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23 AUG 2020

Program Manager or designee, ATCC Federal Solutions

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