

Vector pCAGGS Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike Glycoprotein Gene

Catalog No. NR-52310

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

Product Description:

NR-52310 expresses the full-length, unmodified S glycoprotein, and is intended for producing pseudotyped particles/pseudovirions. NR-52310 is not intended for recombinant protein expression. 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 full-length S sequence for mammalian expression and subcloning into the [pCAGGS](#) mammalian expression vector. NR-52310 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 *E. coli* (Invitrogen™ C404003), 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 vialied in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Lot: 70033697

Manufacturing Date: 13MAR2020

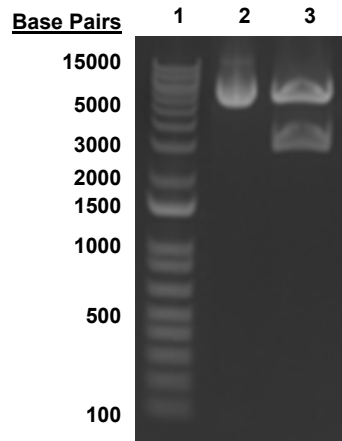
TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing (pre-vial)	~ 8580 base pairs	8585 base pairs ¹
Genotypic Analysis Sequencing of S glycoprotein insert (~ 3820 base pairs)	≥ 99% sequence identity to depositor's sequence	100% sequence identity to depositor's sequence ²
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene <i>bla</i>) ³	<i>bla</i> sequence present	<i>bla</i> sequence present
Agarose Gel Electrophoresis Digestion with <i>ScaI</i>	~ 3 kb and ~ 6 kb	~ 3 kb and ~ 6 kb (Figure 1)
Concentration by PicoGreen® Measurement	≥ 2 µg/mL	1.6 µg in 100 µL per vial (16 µg/mL)
Amount per Vial	Report results	1.6 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	> 500 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-52310 insert was codon optimized for mammalian expression but has a 100% amino acid identity with the SARS-CoV-2, Wuhan-Hu-1 S protein (GenPept: QHD43416).

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.

Figure 1: Agarose Gel of Undigested and Restriction Enzyme Digested NR-52310



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder

Lane 2: NR-52310 undigested

Lane 3: NR-52310 digested

/Heather Couch/

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10 SEP 2020

Program Manager or designee, ATCC Federal Solutions

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