

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

**Catalog No. NR-52309**

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

**Product Description:**

The vector for the receptor binding domain (RBD) of the spike (S) glycoprotein gene from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: [MN908947](#)) was designed by fusing the N-terminal S protein signal sequence to the spike RBD (amino acids 319 to 541) with a C-terminal hexa-histidine tag. The sequence was codon optimized for mammalian expression and subcloned into the [pCAGGS](#) mammalian expression vector. NR-52309 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 vialled in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

**Lot: 70033695**

**Manufacturing Date: 13MAR2020**

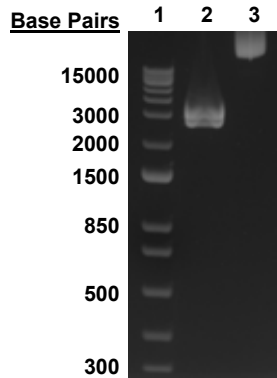
TEST	SPECIFICATIONS	RESULTS
<b>Next-Generation DNA Sequencing (pre-vial)</b>	~ 5490 base pairs	5498 base pairs <sup>1</sup>
<b>Genotypic Analysis</b> Sequencing of spike RBD insert (~ 740 base pairs)	≥ 99% sequence identity to depositor's sequence C-terminal hexa-histidine tag confirmed	100% sequence identity to depositor's sequence <sup>2</sup> C-terminal hexa-histidine tag confirmed
<b>Antibiotic Resistance</b> Ampicillin (encoded by beta-lactamase gene <i>bla</i> ) <sup>3</sup>	<i>bla</i> sequence present	<i>bla</i> sequence present
<b>Agarose Gel Electrophoresis</b> Digestion with <i>ScaI</i> and <i>SacI</i>	~ 2.5 kb and ~ 3 kb	~ 2.5 kb and ~ 3 kb (Figure 1)
<b>Concentration by PicoGreen® Measurement</b>	≥ 2 µg/mL	2.0 µg in 100 µL per vial (20 µg/mL)
<b>Amount per Vial</b>	Report results	2.0 µg per vial
<b>OD<sub>260</sub>/OD<sub>280</sub> Ratio (pre-vial)</b>	1.7 to 2.1	1.9
<b>Effective Bacterial Transformation</b> Invitrogen™ One Shot™ TOP10 <i>E. coli</i>	≥ 50 colonies per ng	> 500 colonies per ng

<sup>1</sup>The sequence was assembled using the depositor's predicted sequence as the reference sequence. The complete plasmid sequence and map are provided on the BEI Resources webpage.

<sup>2</sup>The NR-52309 insert was codon optimized for mammalian expression but has 100% amino acid identity with the SARS-CoV-2, Wuhan-Hu-1 S protein (GenPept: QHD43416).

<sup>3</sup>The 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-52309**



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder  
 Lane 2: NR-52309 digested  
 Lane 3: NR-52309 undigested

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23 MAR 2020

Program Manager or designee, ATCC Federal Solutions

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