

**Vector pCMV/R Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike Glycoprotein Gene**

**Catalog No. NR-53696**

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

**Product Description:**

NR-53696 expresses the full-length, unmodified S glycoprotein, and is intended for producing pseudotyped particles/pseudovirions. NR-53696 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 (residues 1 to 1273) for mammalian expression and subcloning into the pCMV/R mammalian expression vector. The kanamycin resistance gene, *aph*, provides transformant selection through kanamycin 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 kanamycin (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: 70037524**

**Manufacturing Date: 14JUL2020**

TEST	SPECIFICATIONS	RESULTS
<b>Next-Generation DNA Sequencing</b>	~ 8250 base pairs	8249 base pairs <sup>1</sup>
<b>Genotypic Analysis</b> Sequencing of S glycoprotein insert (~ 3800 base pairs)	≥ 99% sequence identity to depositor's sequence	100% sequence identity to depositor's sequence <sup>2</sup>
<b>Antibiotic Resistance</b> Kanamycin (encoded by kanamycin gene <i>aph</i> )	<i>aph</i> sequence present	<i>aph</i> sequence present
<b>Concentration by PicoGreen® Measurement</b>	≥ 2 µg/mL	0.3 µg in 20 µL per vial (14 µg/mL)
<b>Amount per Vial</b>	Report results	0.3 µg per vial
<b>OD<sub>260</sub>/OD<sub>280</sub> Ratio</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	159 colonies per ng

<sup>1</sup>The 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.

<sup>2</sup>The NR-53696 insert was codon optimized, but otherwise is 100% identical to the SARS-CoV-2, Wuhan-Hu-1 S protein (GenPept: YP\_009724390.1).

/Heather Couch/

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18 SEP 2020

Program Manager or designee, ATCC Federal Solutions

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