

**Modified pCAGGS Vector Containing the SARS Coronavirus, Tor2 Non-Structural Protein 6 Gene**

**Catalog No. NR-15194**

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

**Product Description:**

The non-structural protein 6 (nsp6) gene from severe acute respiratory syndrome coronavirus (SARS-CoV), Tor2 (GenBank: [AY274119](#)) was cloned into the modified [pCAGGS](#) mammalian expression vector. pCAGGS was modified by adding a hemagglutinin (HA) tag and 3X FLAG tag C-terminal to the insert coding sequence. NR-15194 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 (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: 70038297**

**Manufacturing Date: 12AUG2020**

TEST	SPECIFICATIONS	RESULTS
<b>Next-Generation DNA Sequencing (pre-vial)</b>	Report results	6546 base pairs <sup>1</sup>
<b>Genotypic Analysis</b> Sequencing of nsp6 insert (870 base pairs)  Sequencing of modified pCAGGS vector (~ 4800 base pairs)	≥ 99% sequence identity to SARS-CoV, Tor2 nsp6 gene (GenBank: AY274119.3) C-terminal HA tag confirmed C-terminal 3X FLAG tag confirmed	100% sequence identity to SARS-CoV, Tor2 nsp6 gene (GenBank: AY274119.3) C-terminal HA tag confirmed C-terminal 3X FLAG tag confirmed
<b>Antibiotic Resistance</b> Ampicillin (encoded by beta-lactamase gene <i>bla</i> ) <sup>2</sup>	<i>bla</i> sequence present	<i>bla</i> sequence present
<b>Concentration by PicoGreen® Measurement</b>	≥ 2 µg/mL	0.4 µg in 30 µL per vial (15 µg/mL)
<b>Amount per Vial</b>	Report results	0.4 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	167 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 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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16 DEC 2020

Program Manager or designee, ATCC Federal Solutions

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