

Modified pCAGGS Vector Containing the SARS Coronavirus, Urbani Non-Structural Protein 3C Gene

Catalog No. NR-15206

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Product Description:

The non-structural protein 3 (nsp3) gene from severe acute respiratory syndrome coronavirus (SARS-CoV), Urbani (GenBank: [AY278741](#)) was designed for expression of a C-terminal NSP3 fragment (residues 1319-1922; NSP3C) and cloned into the modified [pCAGGS](#) mammalian expression vector. pCAGGS was modified by adding a hemagglutinin (HA) tag, as well as a 3X FLAG tag C-terminal to the insert coding sequence. NR-15206 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: 70038306

Manufacturing Date: 12AUG2020

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing (pre-vial)	Report results	7657 base pairs ¹
Genotypic Analysis Sequencing of nsp3 insert (~ 1810 base pairs) Sequencing of modified pCAGGS vector (~ 4800 base pairs)	≥ 99% sequence identity to SARS-CoV, Urbani nsp3 gene (GenBank: AY278741.1) C-terminal HA tag confirmed C-terminal 3X Flag tag confirmed	100% sequence identity to SARS-CoV, Urbani nsp3 gene (GenBank: AY278741.1) ² C-terminal HA tag confirmed C-terminal 3X Flag tag confirmed
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene <i>bla</i>) ³	<i>bla</i> sequence present	<i>bla</i> sequence present
Concentration by PicoGreen® Measurement	≥ 2 µg/mL	0.4 µg in 20 µL per vial (22 µg/mL)
Amount per Vial	Report results	0.4 µg per vial
OD₂₆₀/OD₂₈₀ Ratio	1.7 to 2.1	1.9
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 <i>E. coli</i>	≥ 50 colonies per ng	90 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.

²Residues 6673-8484

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.

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Program Manager or designee, ATCC Federal Solutions

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