Certificate of Analysis for NR-15203

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

Catalog No. NR-15203

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

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 an N-terminal NSP3 fragment (residues 1-1318) and cloned into the modified pCAGGS mammalian expression vector. pCAGGS was modified by adding a hemagglutinin (HA) tag, as well as a tobacco etch virus (TEV) cleavable BirA biotinylation tag C-terminal to the insert coding sequence. NR-15203 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 vialed in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Lot: 70038300 **Manufacturing Date: 12AUG2020**

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing (pre-vial)	Report results	9780 base pairs[1]
Genotypic Analysis Sequencing of nsp3 insert (~ 3950 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 TEV protease cleavage site confirmed C-terminal BirA biotinylation tag confirmed	100% sequence identity to SARS-CoV, Urbani nsp3 gene (GenBank: AY278741.1)[2] C-terminal HA tag confirmed C-terminal TEV protease cleavage site confirmed C-terminal BirA biotinylation tag confirmed
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene *bla*)[3]	*bla* sequence present	*bla* sequence present
Concentration by PicoGreen® Measurement	≥ 2 µg/mL	0.4 µg in 20 µL per vial (20 µg/mL)
Amount per Vial	Report results	0.4 µg per vial
OD_{260}/OD_{280} Ratio	1.7 to 2.1	1.9
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 *E. coli*	≥ 50 colonies per ng	131 colonies per ng

[1]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.
[2]Residues 819 to 2136
[3]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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Program Manager or designee, ATCC Federal Solutions

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Support Provided by NIAID

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