b|e|i RESOURCES

SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-15204

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

Catalog No. NR-15204

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 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 tobacco etch virus (TEV) cleavable BirA biotinylation tag C-terminal to the insert coding sequence. NR-15204 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: 70038303 **Manufacturing Date: 12AUG2020**

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing (pre-vial)	Report results	7638 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 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	133 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 6673-8484
[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/
Heather Couch 14 DEC 2020
Program Manager or designee, ATCC Federal Solutions

ATCC®, on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected by ATCC® and the contributor to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC®'s knowledge.

ATCC® is a trademark of the American Type Culture Collection.
You are authorized to use this product for research use only. It is not intended for human use.

Support Provided by NIAID

BEI Resources
www.beiresources.org
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898