Certificate of Analysis for NR-54006

Vector pCAGGS Containing the SARS-Related Coronavirus 2, Alpha Variant Spike Glycoprotein Receptor Binding Domain (RBD) Gene

Catalog No. NR-54006

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

Product Description:

The vector for the receptor binding domain (RBD) of the spike (S) glycoprotein gene from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: MN908947) was designed by fusing the N-terminal S protein signal sequence to the spike RBD (amino acids 319 to 541) with a C-terminal hexa-histidine tag. The sequence was codon optimized for mammalian expression, mutated to include the Alpha variant [also referred to as the United Kingdom (UK) variant; B.1.1.7 lineage] N501Y mutation and subcloned into the pCAGGS mammalian expression vector. NR-54006 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).

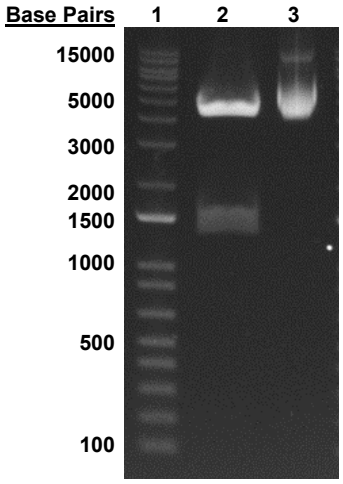
Lot: 70041663 **Manufacturing Date: 26JAN2021**

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 5490 base pairs	5497 base pairs[1]
Genotypic Analysis Sequencing of spike RBD insert (~ 740 base pairs)	≥ 99% sequence identity to depositor's sequence C-terminal hexa-histidine tag confirmed	100% sequence identity to depositor's sequence[2] C-terminal hexa-histidine tag confirmed
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene *bla*)[3]	*bla* sequence present	*bla* sequence present
Agarose Gel Electrophoresis Digestion with *Hind*III and *Xba*I	~ 1.5 kb and ~ 4 kb	~ 1.5 kb and ~ 4 kb
Concentration by PicoGreen® Measurement	≥ 2 µg/mL	0.3 µg in 20 µL per vial (16 µg/mL)
Amount per Vial	Report results	0.3 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	284 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]The NR-54006 insert was codon optimized for mammalian expression but has 100% amino acid identity with the SARS-CoV-2, Wuhan-Hu-1 S protein (GenPept: QHD43416) other than the alpha variant mutation.
[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.

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

Figure 1: Agarose Gel of Undigested and Restriction Enzyme Digested NR-54006



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
Lane 2: NR-54006 digested
Lane 3: NR-54006 undigested

/Heather Couch/
Heather Couch 06 AUG 2021
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 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.

Support Provided by NIAID

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.

BEI Resources
www.beiresources.org

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