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SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-52309

Vector pCAGGS Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike Glycoprotein Gene Receptor Binding Domain (RBD)

Catalog No. NR-52309

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 and subcloned into the pCAGGS mammalian expression vector. NR-52309 contains the beta-lactamase gene, *bla*, to provide transformant selection through ampicillin resistance in *Escherichia coli (E. coli)*. Lot 70036315 was produced from a preparation of glycerol stock (NRC-52309 lot 70033696), grown in Luria-Bertani broth with ampicillin (50 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: 70036315 **Manufacturing Date: 28MAY2020**

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing[1]	~ 5490 base pairs	5498 base pairs
Genotypic Analysis[1] 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 C-terminal hexa-histidine tag confirmed
Antibiotic Resistance[1] Ampicillin (encoded by beta-lactamase gene *bla*)[2]	*bla* sequence present	*bla* sequence present
Agarose Gel Electrophoresis Digestion with *Sca*I and *Sac*I	~ 2.5 kb and ~ 3 kb	~ 2.5 kb and ~ 3 kb
Concentration by Picogreen® Measurement	≥ 2 µg/mL	0.9 µg in 100 µL per vial (8.6 µg/mL)
Amount per Vial	Report results	0.9 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	111 colonies per ng

[1]This test was performed pre-vial on a previous lot of extracted material (NR-52309 lot 70033695).
[2]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 08 JUN 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 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

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