SUPPORTING INFECTIOUS DISEASE RESEARCH

Vector pCAGGS Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike Glycoprotein Gene

Catalog No. NR-52310

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

Product Description:

NR-52310 expresses the full-length, unmodified S glycoprotein, and is intended for producing pseudotyped particles/pseudovirions. NR-52310 is not intended for recombinant protein expression. The vector for the spike (S) glycoprotein gene from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: <u>MN908947</u>) was designed by codon optimizing the full-length S sequence for mammalian expression and subcloning into the <u>pCAGGS</u> mammalian expression vector. NR-52310 contains the beta-lactamase gene, *bla*, to provide transformant selection through ampicillin resistance in *Escherichia coli (E. coli)*. Lot 70034637 was produced from a preparation of glycerol stock (NRC-52310 lot 70033698), 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: 70034637

Manufacturing Date: 09APR2020

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing ¹	~ 8580 base pairs	8585 base pairs
Genotypic Analysis ¹ Sequencing of S glycoprotein insert (~ 3820 base pairs)	≥ 99% sequence identity to depositor's sequence	100% sequence identity to depositor's sequence
Antibiotic Resistance ¹ Ampicillin (encoded by beta-lactamase gene <i>bla</i>) ²	<i>bla</i> sequence present	<i>bla</i> sequence present
Agarose Gel Electrophoresis Digestion with Sapl and Sacl	~ 1 kb and ~ 7 kb	~ 1 kb and ~ 7 kb (Figure 1)
Concentration by Picogreen [®] Measurement	≥ 2 µg/mL	1.2 μg in 100 μL per vial (12 μg/mL)
Amount per Vial	Report results	1.2 μg per vial
OD ₂₆₀ /OD ₂₈₀ Ratio (pre-vial)	1.7 to 2.1	2.0
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 <i>E. coli</i>	≥ 50 colonies per ng	Not determined

¹This test was performed pre-vial on a previous lot of extracted material (NR-52310 lot 70033697).

²The 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.

b|**e**|**i** resources

SUPPORTING INFECTIOUS DISEASE RESEARCH

Base Pairs 1 2 3 15000 5000 5000 5000 3000 2000 5000 5000 1500 1000 5000 5000 1000 500 1000 5000 Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder Lane 2: NR-52310 undigested

Figure 1: Agarose Gel of Undigested and Restriction Enzyme Digested NR-52310

/Heather Couch/ _<u>Heather C</u>ouch

21 APR 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.

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.

Lane 3: NR-52310 digested