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 70036316 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: 70036316

Manufacturing Date: 28MAY2020

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 Scal	~ 3 kb and ~ 6 kb	~ 3 kb and ~ 6 kb (Figure 1)
Concentration by Picogreen® Measurement	≥ 2 µg/mL	0.6 μg in 100 μL per vial (5.6 μg/mL)
Amount per Vial	Report results	0.6 µg per vial
OD ₂₆₀ /OD ₂₈₀ Ratio (pre-vial)	1.7 to 2.1	1.9
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 <i>E. coli</i>	≥ 50 colonies per ng	248 colonies per ng

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.

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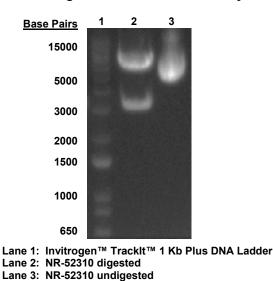


Figure 1: Agarose Gel of Undigested and Restriction Enzyme Digested NR-52310

/Heather Couch/ Heather Couch

Program Manager or designee, ATCC Federal Solutions

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11 JUN 2020