Certificate of Analysis for NR-52394

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

Catalog No. NR-52394

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

Product Description:

The vector for the spike (S) glycoprotein gene from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: MN908947) was designed for expression of a soluble S glycoprotein with a polybasic cleavage site deletion (RRAR to A; residues 682 to 685) and stabilizing mutations (K986P and V987P, wild type numbering) with a C-terminal thrombin cleavage site, T4 foldon trimerization domain and hexa-histidine tag. The S sequence was codon optimized for mammalian expression and subcloned into the pCAGGS mammalian expression vector. NR-52394 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 (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: 70034717 **Manufacturing Date: 03APR2020**

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 8530 base pairs	8537 base pairs[1]
Genotypic Analysis Sequencing of S glycoprotein insert (~ 3770 base pairs)	100% sequence identity to depositor's sequence Thrombin protease site sequence confirmed T4 foldon trimerization domain sequence confirmed His_6 tag sequence confirmed	100% sequence identity to depositor's sequence[2] Thrombin protease site sequence confirmed T4 foldon trimerization domain sequence confirmed His_6 tag sequence confirmed
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene *bla*)[3]	*bla* sequence present	*bla* sequence present
Agarose Gel Electrophoresis Digestion with *Sca*I	~ 6 kb and ~ 3 kb	~ 6 kb and ~ 3 kb (Figure 1)
Concentration by PicoGreen® Measurement	≥ 2 µg/mL	0.6 µg in 100 µL per vial (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	2.0
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 *E. coli*	≥ 50 colonies per ng	> 500 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-52394 insert was codon optimized for mammalian expression with mutations for stability and solubility, but otherwise is consistent with the SARS-CoV-2, Wuhan-Hu-1 S protein (GenPept: QHD43416).
[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.

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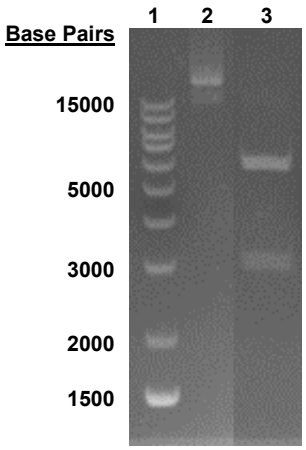
Figure 1: Agarose Gel of Undigested and Restriction Enzyme Digested NR-52394



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
Lane 2: NR-52394 undigested
Lane 3: NR-52394 digested

/Heather Couch/
Heather Couch 24 AUG 2020
Program Manager or designee, ATCC Federal Solutions

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Support Provided by NIAID

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