

Certificate of Analysis for NR-53508

Vector pET-28a(+) Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Membrane Glycoprotein Gene

Catalog No. NR-53508

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

Product Description:

The membrane glycoprotein (M) gene from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: MN908947) was codon optimized, tagged with a tobacco etch virus (TEV) cleavable N-terminal hexa-histidine tag and cloned into the pET-28a(+) plasmid. The kanamycin resistance gene, *aph*, provides transformant selection through kanamycin 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 kanamycin (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: 70036473 Manufacturing Date: 04JUN2020

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 5980 base pairs	5970 base pairs ¹
Genotypic Analysis Sequencing of M insert (~ 670 base pairs)	100% sequence identity to depositor's sequence His ₆ tag sequence confirmed TEV protease site sequence confirmed	100% sequence identity to depositor's sequence ² His ₆ tag sequence confirmed TEV protease site sequence confirmed
Antibiotic Resistance Kanamycin (encoded by <i>aph</i>)	aph sequence present	aph sequence present
Concentration by Qubit™ Measurement	≥ 2 µg/mL	0.2 μg in 20 μL per vial (8 μg/mL)
Amount per Vial	Report results	0.2 μg per vial
OD ₂₆₀ /OD ₂₈₀ Ratio	1.7 to 2.1	1.9
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 <i>E. coli</i>	≥ 50 colonies per ng	301 colonies per ng

¹The 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.

/Heather Couch/

Heather Couch 26 AUG 2020

Program Manager or designee, ATCC Federal Solutions

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²The NR-53508 insert was codon optimized but otherwise is 100% identical with the SARS-CoV-2, Wuhan-Hu-1 M protein (GenPept: QHD43419.1).