

Certificate of Analysis for NR-53502

Vector pET-28a(+) Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Non-Structural Protein 10 Gene

Catalog No. NR-53502

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

Product Description:

The non-structural protein 10 (nsp10) 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: 70036467 Manufacturing Date: 03JUN2020

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 5730 base pairs	5724 base pairs ¹
Genotypic Analysis Sequencing of nsp10 insert (~ 420 base pairs)	100% sequence identity to depositor's sequence His₀ 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 aph)	aph sequence present	aph sequence present
Concentration by Qubit™ Measurement	≥ 2 µg/mL	0.3 μg in 20 μL per vial (14 μg/mL)
Amount per Vial	Report results	0.3 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	133 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 14 AUG 2020

Program Manager or designee, ATCC Federal Solutions

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²The NR-53502 insert was codon optimized but otherwise is 100% identical with the SARS-CoV-2, Wuhan-Hu-1 NSP10 protein (GenPept: QHD43415).