

Certificate of Analysis for NR-53500

Vector pET-28a(+) Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Non-Structural Protein 8 Gene

Catalog No. NR-53500

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

Product Description:

The non-structural protein 8 (nsp8) gene from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: MN908947) was codon optimized, tagged with a thrombin 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-HCI, 1 mM EDTA, pH 8.0).

Lot: 70036465 Manufacturing Date: 03JUN2020

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 5890 base pairs	5898 base pairs¹
Genotypic Analysis Sequencing of nsp8 insert (~ 590 base pairs)	100% sequence identity to depositor's sequence His6 tag sequence confirmed Thrombin cleavage site sequence confirmed	100% sequence identity to depositor's sequence ² His ₆ tag sequence confirmed Thrombin cleavage 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 (10 μg/mL)
Amount per Vial	Report results	0.2 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	176 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-53500 insert was codon optimized but is 100% identical with the SARS-CoV-2, Wuhan-Hu-1 NSP8 protein (GenPept: QHD43415).