

Vector pET-28a(+) Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Non-Structural Protein 15 Gene

Catalog No. NR-53506

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Product Description:

The non-structural protein 15 (nsp15) 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 vialled in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Lot: 70036471

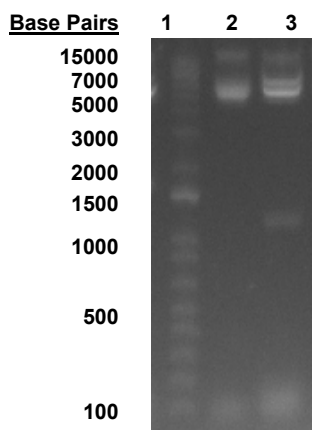
Manufacturing Date: 04JUN2020

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 6350 base pairs	6345 base pairs ¹
Genotypic Analysis Sequencing of nsp15 insert (~ 1040 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>)	<i>aph</i> sequence present	<i>aph</i> sequence present
Agarose Gel Electrophoresis Digestion with <i>Xho</i> I and <i>Xba</i> I (pre-vial)	~ 5 kb and ~ 1.2 kb	~ 5 kb and ~ 1.2 kb (Figure 1)
Concentration by Qubit™ Measurement	≥ 2 µg/mL	0.3 µg in 20 µL per vial (15 µg/mL)
Amount per Vial	Report results	0.3 µg per vial
OD₂₆₀/OD₂₈₀ Ratio	1.7 to 2.1	1.9
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 <i>Escherichia coli</i>	≥ 50 colonies per ng	204 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.

²The NR-53506 insert was codon optimized but otherwise is 100% identical with the SARS-CoV-2, Wuhan-Hu-1 NSP15 protein (GenPept: QHD43415).

Figure 1: Agarose Gel of Undigested and Restriction Enzyme Digested NR-53506



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
 Lane 2: NR-53506 undigested
 Lane 3: NR-53506 digested

/Heather Couch/
 Heather Couch

25 AUG 2020

Program Manager or designee, ATCC Federal Solutions

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