

Certificate of Analysis for NR-52432

Vector pET-11a Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Non-Structural Protein 7 Gene

Catalog No. NR-52432

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

Product Description:

The non-structural protein 7 (nsp7) 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-11a plasmid. The beta-lactamase gene, *bla*, provides 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: 70035134 Manufacturing Date: 13MAY2020

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	Report results	5995 base pairs¹
Genotypic Analysis Sequencing of nsp7 insert (~ 350 base pairs)	100% sequence identity to depositor's sequence Hise 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 Ampicillin (encoded by beta-lactamase gene bla) ³	bla sequence present	bla sequence present
Concentration by PicoGreen® Measurement	≥ 2 µg/mL	0.1 μg in 20 μL per vial (7 μg/mL)
Amount per Vial	Report results	0.1 μg per vial
OD ₂₆₀ /OD ₂₈₀ Ratio	1.7 to 2.1	1.8
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 <i>E. coli</i>	≥ 50 colonies per ng	> 500 colonies per ng

¹The sequence was assembled pre-vial using the predicted sequence as the reference sequence. The complete plasmid sequence and map are provided on the BEI Resources webpage.

/Heather Couch/

Heather Couch 10 JUN 2020

Program Manager or designee, ATCC Federal Solutions

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²The NR-52432 insert was codon optimized but is 100% identical with the SARS-CoV-2, Wuhan-Hu-1 NSP7 protein (GenPept: YP_009725303.1).

³The 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.