

Vector pET-28a(+) Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Open Reading Frame 3a Gene

Catalog No. NR-53509

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Product Description:

The open reading frame 3a (orf3a) 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 vialied in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Lot: 70036474

Manufacturing Date: 04JUN2020

| TEST | SPECIFICATIONS | RESULTS |
|---|---|--|
| Next-Generation DNA Sequencing | ~ 6140 base pairs | 6129 base pairs ¹ |
| Genotypic Analysis Sequencing of orf3a insert (~ 830 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 |
| Concentration by Qubit™ Measurement | ≥ 2 µg/mL | 0.1 µg in 20 µL per vial (6 µg/mL) |
| Amount per Vial | Report results | 0.1 µg per vial |
| OD₂₆₀/OD₂₈₀ Ratio | 1.7 to 2.1 | 1.8 |
| Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 <i>E. coli</i> | ≥ 50 colonies per ng | 286 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-53509 insert was codon optimized but otherwise is 100% identical with the SARS-CoV-2, Wuhan-Hu-1 ORF3a protein (GenPept: QHD43417.1).

/Heather Couch/
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27 AUG 2020

Program Manager or designee, ATCC Federal Solutions

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