

## **Certificate of Analysis for NR-52437**

# Vector pET-11a Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 3C-Like Protease Gene

#### Catalog No. NR-52437

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

#### **Product Description:**

The 3C-like protease (3LCpro) from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: MN908947) was codon optimized, tagged with an N-terminal hexa-histidine tag followed by a 3CLpro auto cleavage site 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: 70036756 Manufacturing Date: 06MAY2020

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	Report results	6598 base pairs <sup>1</sup>
Genotypic Analysis Sequencing of 3Cpro insert (~ 1070 base pairs)	100% sequence identity to depositor's sequence Hise tag sequence confirmed 3CLpro auto cleavage site sequence confirmed	100% sequence identity to depositor's sequence <sup>2</sup> His <sub>6</sub> tag sequence confirmed 3CLpro auto cleavage site sequence confirmed
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene bla) <sup>3</sup>	bla sequence present	bla sequence present
Concentration by PicoGreen® Measurement	≥ 2 µg per mL	0.2 μg in 25 μL per vial (6 μg per mL)
Amount per Vial	Report results	0.2 μg per vial
OD <sub>260</sub> /OD <sub>280</sub> Ratio	1.7 to 2.1	1.9
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 <i>E. coli</i>	≥ 50 colonies per ng	> 500 colonies per ng

<sup>&</sup>lt;sup>1</sup>The 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 06 APR 2021

Program Manager or designee, ATCC Federal Solutions

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<sup>&</sup>lt;sup>2</sup>The NR-52437 insert was codon optimized but is consistent with the SARS-CoV-2, Wuhan-Hu-1 3Cpro protein (GenPept: YP\_009742610.1).

<sup>&</sup>lt;sup>3</sup>The 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.