

**Vector pLVX-EF1α-IRES-Puro Containing the SARS-Related Coronavirus 2, USA-WA1/2020 Non-Structural Protein 7 Gene**

**Catalog No. NR-52955**

**Product Description:**

Note: The vial label indicates this product contains a TST tag. This nomenclature refers to a 2X Strep tag. This product does not express the Twin-Strep-tag® that is commonly referred to as a TST tag. The non-structural protein 7 (nsp7) gene from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), USA-WA1/2020 (GenBank: [MN985325](#)) was codon optimized and modified by the addition of C-terminal 2X Strep tag and cloned into the [pLVX-EF1α-IRES-Puro](#) lentiviral expression plasmid. The vector contains an internal ribosomal entry site (IRES) that allows a gene-of-interest and a puromycin resistance gene to be simultaneously co-expressed from a single mRNA transcript. Expression of the transcript is driven by the human elongation factor 1 alpha (EF1α) promoter. The beta-lactamase gene, *bla*, provides transformant selection through ampicillin resistance in *Escherichia coli* (*E. coli*) and the puromycin resistance gene, *pac*, provides transformant selection through puromycin resistance in eukaryotic cells. The deposited plasmid was transformed into NEB® Stable Competent *E. coli* cells (New England Biolabs® C3040H), grown in Luria-Bertani broth with ampicillin (100 µg per mL) for 1 day at 30°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: 70037531**

**Manufacturing Date: 14JUL2020**

| TEST  | SPECIFICATIONS   | RESULTS   |
|---|--|---|
| <b>Next-Generation DNA Sequencing</b>   | ~ 9170 base pairs  | 9165 base pairs <sup>1</sup>  |
| <b>Genotypic Analysis</b><br>Sequencing of nsp7 insert (~ 250 base pairs)   | ≥ 99% sequence identity to depositor's sequence<br>2X Strep tag sequence confirmed | 100% sequence identity to depositor's sequence <sup>2</sup><br>2X Strep tag sequence confirmed <sup>3</sup> |
| <b>Antibiotic Resistance</b><br>Ampicillin (encoded by beta-lactamase gene <i>bla</i> ) <sup>4</sup><br>Puromycin (encoded by puromycin n-acetyltransferase gene <i>pac</i> ) | <i>bla</i> sequence present<br><i>pac</i> sequence present                         | <i>bla</i> sequence present<br><i>pac</i> sequence present  |
| <b>Concentration by PicoGreen® Measurement</b>  | ≥ 2 µg/mL  | 0.2 µg in 20 µL per vial (8 µg/mL)  |
| <b>Amount per Vial</b>  | Report results   | 0.2 µg per vial   |
| <b>OD<sub>260</sub>/OD<sub>280</sub> Ratio</b>  | 1.7 to 2.1   | 1.9   |
| <b>Effective Bacterial Transformation</b><br>NEB® Stable Competent <i>E. coli</i> cells   | ≥ 50 colonies per ng   | 298 colonies per ng   |

<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.

<sup>2</sup>The NR-52955 insert was codon optimized but is 100% identical with the SARS-CoV-2, USA-WA1/2020 ORF1ab polyprotein (GenPept QHO60603.1).

<sup>3</sup>This TST tag is a tandem Strep tag, defined by the sequence N-WSHPQFEKGGGSGGGSGGGSWHPQFEK-C. For more information, please see Gordon, D. E., et al. "A SARS-CoV-2 Protein Interaction Map Reveals Targets for Drug Repurposing." *Nature* 583 (2020): 459-468. PubMed: 32353859.

<sup>4</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.

/Heather Couch/

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06 OCT 2020

Program Manager or designee, ATCC Federal Solutions

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