

**Vector pLVX-EF1 $\alpha$ -IRES-Puro Containing the SARS-Related Coronavirus 2, USA-WA1/2020 Open Reading Frame 8 Gene**

Catalog No. NR-52972

**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<sup>®</sup> that is commonly referred to as a TST tag. The open reading frame 8 (orf8) 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 a C-terminal 2X Strep tag and cloned into the [pLVX-EF1 \$\alpha\$ -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 (EF1 $\alpha$ ) 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<sup>®</sup> Stable Competent *E. coli* cells (New England Biolabs<sup>®</sup> C3040H), grown in Luria-Bertani broth with ampicillin (100  $\mu$ g per mL) for 1 day at 30°C in an aerobic atmosphere, extracted using a Plasmid *Plus* Maxi Kit (QIAGEN<sup>®</sup> 12963) and vialled in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Lot: 70037926

Manufacturing Date: 05AUG2020

| TEST   | SPECIFICATIONS   | RESULTS   |
|--|--|---|
| Next-Generation DNA Sequencing   | ~ 9280 base pairs  | 9276 base pairs <sup>1</sup>  |
| Genotypic Analysis<br>Sequencing of orf8 insert<br>(~ 360 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> |
| Antibiotic Resistance<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  |
| Concentration by PicoGreen <sup>®</sup> Measurement  | ≥ 2 $\mu$ g/mL   | 0.4 $\mu$ g in 20 $\mu$ L per vial (18 $\mu$ g/mL)  |
| Amount per Vial  | Report results   | 0.4 $\mu$ g per vial  |
| OD <sub>260</sub> /OD <sub>280</sub> Ratio (pre-vial)  | 1.7 to 2.1   | 1.9   |
| Effective Bacterial Transformation<br>NEB <sup>®</sup> Stable Competent <i>E. coli</i>   | ≥ 50 colonies per ng   | 111 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-52972 insert was codon optimized but is 100% identical with the SARS-CoV-2 ORF8 protein (GenPept: QHO60600.1).

<sup>3</sup>This 2X Strep tag is defined by the sequence N-WSHPQFEKGGGSGGGSGGGWSHPQFEK-C. For more information, please see Busby, M., et al. "Optimisation of a Multivalent Strep Tag for Protein Detection." *Biophys. Chem.* 152 (2010): 170-177. PubMed: 20970240.

<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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20 OCT 2020

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