

Vector pLVX-EF1 α -IRES-Puro Containing the SARS-Related Coronavirus 2, USA-WA1/2020 Non-Structural Protein 8 Gene

Catalog No. NR-52956

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 8 (nsp8) 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 α) 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 μ g 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: 70037532

Manufacturing Date: 14JUL2020

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 9510 base pairs	9510 base pairs ¹
Genotypic Analysis Sequencing of nsp8 insert (~ 600 base pairs)	≥ 99% sequence identity to depositor's sequence 2X Strep tag sequence confirmed	100% sequence identity to depositor's sequence ² 2X Strep tag sequence confirmed ³
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene <i>bla</i>) ⁴ Puromycin (encoded by puromycin n-acetyltransferase gene <i>pac</i>)	<i>bla</i> sequence present <i>pac</i> sequence present	<i>bla</i> sequence present <i>pac</i> sequence present
Concentration by PicoGreen [®] Measurement	≥ 2 μ g/mL	0.2 μ g in 20 μ L per vial (8 μ g/mL)
Amount per Vial	Report results	0.2 μ g per vial
OD ₂₆₀ /OD ₂₈₀ Ratio	1.7 to 2.1	2.0
Effective Bacterial Transformation NEB [®] Stable Competent <i>E. coli</i> cells	≥ 50 colonies per ng	307 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.

²The NR-52956 insert was codon optimized but is 100% identical with the SARS-CoV-2, USA-WA1/2020 ORF1ab polyprotein (GenPept: QHO60603.1).

³This 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.

⁴The 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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08 OCT 2020

Program Manager or designee, ATCC Federal Solutions

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