

Vector pLVX-EF1α-IRES-Puro Containing the SARS-Related Coronavirus 2, USA-WA1/2020 Open Reading Frame 9c Gene

Catalog No. NR-52975

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 open reading frame 9c (orf9c) 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 N-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: 70037930

Manufacturing Date: 05AUG2020

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 9130 base pairs	9126 base pairs ¹
Genotypic Analysis Sequencing of orf9c insert (~ 220 base pairs)	100% 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 µg/mL	0.26 µg in 20 µL per vial (13 µg/mL)
Amount per Vial	Report results	0.26 µg per vial
OD ₂₆₀ /OD ₂₈₀ Ratio (pre-vial)	1.7 to 2.1	1.9
Effective Bacterial Transformation NEB® Stable Competent <i>E. coli</i>	≥ 50 colonies per ng	102 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-52975 insert was codon optimized but is 100% identical with the SARS-CoV-2 ORF9c protein (UniProtKB: P0DTD3.1).

³This 2X Strep tag is defined by the sequence N-WSHPQFEKGGSGGGSGGGSGGGWSHPQFEK-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.

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.

Certificate of Analysis for NR-52975**/Heather Couch/****Heather Couch**

16 OCT 2020

Program Manager or designee, ATCC Federal Solutions

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