

Plasmid pUC57-Simple Containing cDNA from Enterovirus D68, EV-D68/USA/2018-23088, Infectious Clone EV-D68-R23088

Catalog No. NR-52379

Product Description:

The enterovirus species D type 68 (EV-D68), EV-D68/USA/2018-23088 (GenBank: [MN245982](#)) genome was cloned into the *Escherichia coli* (*E. coli*) cloning vector [pUC57-simple](#) to generate plasmid EV-D68-R23088. EV-D68-R23088 contains a T7 bacteriophage promoter immediately upstream of the 5' end of the viral genome. Transfection of cells with RNA transcribed *in vitro* from the linearized plasmid results in production of infectious virus particles. EV-D68-R23088 also contains the beta-lactamase gene, *bla*, to provide transformant selection through ampicillin resistance in *E. coli*. The deposited plasmid was transformed into NEB® Stable Competent *E. coli* cells (New England Biolabs® C3040H), grown in Luria-Bertani broth containing 50 µg per mL ampicillin for 1 day at 37°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: 70035765

Manufacturing Date: 13MAY2020

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~10,120 base pairs	10,118 base pairs ¹
Genotypic Analysis Sequencing of Enterovirus D68 insert (~ 7320 base pairs)	≥ 99% sequence identity to EV-D68/USA/2018-23088 (GenBank: MN245982.1)	100% sequence identity to EV-D68/USA/2018-23088 (GenBank: MN245982.1)
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene <i>bla</i>) ²	<i>bla</i> sequence present	<i>bla</i> sequence present
Concentration by PicoGreen® Measurement	≥ 2 µg/mL	0.2 µg in 100 µL (2 µg/mL)
Amount per Vial	Report results	0.2 µg per vial
OD₂₆₀/OD₂₈₀ Ratio (pre-vial)	1.7 to 2.1	1.8
Effective Bacterial Transformation NEB® Stable Competent <i>E. coli</i>	≥ 50 colonies per ng	274 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 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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21 SEP 2020

Program Manager or designee, ATCC Federal Solutions

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