

Plasmid pUC57-Simple Containing cDNA from Enterovirus D68, USA/WI/2009-23230, Infectious Clone EV-D68-R23230

Catalog No. NR-52377

Product Description:

The enterovirus species D type 68 (EV-D68), USA/WI/2009-23230 (GenBank: [MN240506](#)) genome was cloned into the *Escherichia coli* (*E. coli*) cloning vector [pUC57-simple](#) to generate plasmid EV-D68-R23230. EV-D68-R23230 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-R23230 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: 70036104

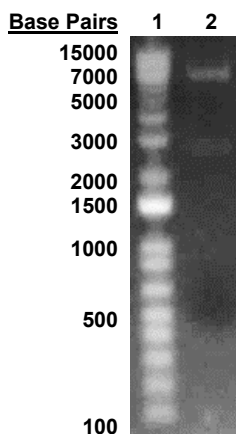
Manufacturing Date: 28MAY2020

| TEST | SPECIFICATIONS | RESULTS |
|--|--|---|
| Next-Generation DNA Sequencing | ~10,130 base pairs | 10,127 base pairs ¹ |
| Genotypic Analysis Sequencing of Enterovirus D68 insert (~7340 base pairs) | ≥ 99% sequence identity to EV-D68, USA/WI/2009-23230 (GenBank: MN240506.1) | 100% sequence identity to EV-D68, USA/WI/2009-23230 (GenBank: MN240506.1) |
| Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene <i>bla</i>) ² | <i>bla</i> sequence present | <i>bla</i> sequence present |
| Agarose Gel Electrophoresis Digestion with <i>Xho</i> I and <i>Not</i> I | ~ 7 kb and ~3 kb | ~ 7 kb and ~ 3 kb (Figure 1) |
| Concentration by PicoGreen® Measurement | ≥ 2 µg/mL | 0.7 µg in 100 µL per vial (7 µg/mL) |
| Amount per Vial | Report results | 0.7 µg per vial |
| OD₂₆₀/OD₂₈₀ Ratio | 1.7 to 2.1 | 1.9 |
| Effective Bacterial Transformation NEB® Stable Competent <i>E. coli</i> | ≥ 50 colonies per ng | 106 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.

Figure 1: Agarose Gel of Restriction Enzyme Digested NR-52377



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
 Lane 2: NR-52377 digested

/Heather Couch/

Heather Couch

05 SEP 2020

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