

Vector pHDM Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike Glycoprotein Ectodomain Mutant, HA Tag

Catalog No. NR-52513

Product Description:

The vector for the spike (S) glycoprotein gene from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: [NC_045512](#)) was designed by codon optimizing the S sequence (residues 1 to 1239) for mammalian expression fused to the C-terminus of the hemagglutinin gene (residues 555 to 565) from Influenza A/WSN/1933 (H1N1) and subcloned into the pHDM vector under the CMV promoter. NR-52513 contains the beta-lactamase gene, *bla*, to provide transformant selection through ampicillin resistance in *Escherichia coli* (*E. coli*). The deposited plasmid was transformed into One Shot™ TOP10 *E. coli* (Invitrogen™ C404003), grown in Luria-Bertani broth with ampicillin (50 µg per mL) 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: 70035470

Manufacturing Date: 29APR2020

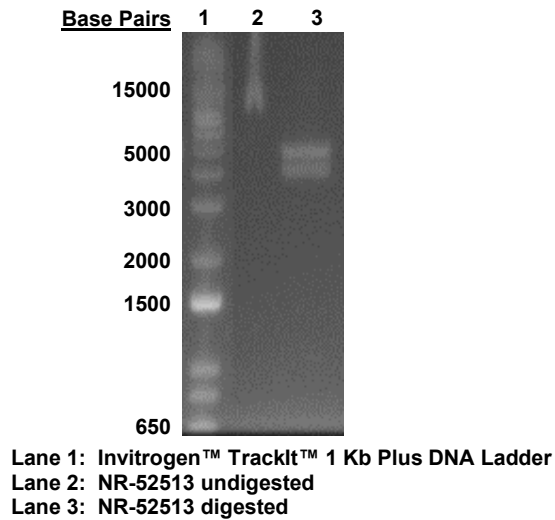
| TEST | SPECIFICATIONS | RESULTS |
|--|---|--|
| Next-Generation DNA Sequencing | ~ 8310 base pairs | 8311 base pairs ¹ |
| Genotypic Analysis Sequencing of S glycoprotein insert (~ 3720 base pairs) | 100% sequence identity to depositor's sequence C-terminal HA tag confirmed | 100% sequence identity to depositor's sequence ² C-terminal HA tag confirmed |
| Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene <i>bla</i>) ³ | <i>bla</i> sequence present | <i>bla</i> sequence present |
| Agarose Gel Electrophoresis (pre-vial) Digestion with <i>SapI</i> | ~ 5 kb and ~ 4 kb | ~ 5 kb and ~ 4 kb (Figure 1) |
| Concentration by PicoGreen® Measurement | ≥ 2 µg/mL | 0.2 µg in 20 µL per vial (8 µg/mL) |
| Amount per Vial | Report results | 0.2 µg per vial |
| OD₂₆₀/OD₂₈₀ Ratio | 1.7 to 2.1 | 1.9 |
| Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 <i>E. coli</i> | ≥ 50 colonies per ng | 156 colonies per ng |

¹The sequence was assembled pre-vial using the depositor's predicted sequence as the reference sequence. The complete plasmid sequence and map are provided on the BEI Resources webpage.

²NR-52513 insert was codon optimized for mammalian expression, but otherwise is 100% identical to the SARS-CoV-2, Wuhan-Hu-1 S protein (GenPept: YP_009724390; residues 1-1239).

³The antibiotic ampicillin degrades quickly during growth. Bacterial stationary phase should be minimized during plasmid replication to avoid plasmid loss and increased antibiotic concentrations may be necessary.

Figure 1: Agarose Gel of Undigested and Restriction Enzyme Digested NR-52513



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25 JAN 2021

Program Manager or designee, ATCC Federal Solutions

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