

Modified pαH Vector Containing the Human Angiotensin-Converting Enzyme 2

Catalog No. NR-52565

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Product Description:

The vector for the human angiotensin-converting enzyme 2 (ACE2) (GenBank: [GQ262784](#)) was designed by subcloning the ACE2 mRNA sequence into the pαH mammalian expression vector, which was modified by subcloning an HRV3C protease cleavage site, and the tags octa-histidine and *Strep-tag*[®] II downstream of the open reading frame. NR-52565 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 *Escherichia coli* (Invitrogen[™] C404010), 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: 70035522

Manufacturing Date: 01MAY2020

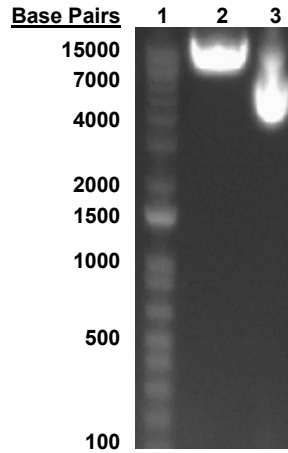
TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing (pre-vial)	Report results	7011 base pairs ¹
Genotypic Analysis Sequencing of ACE2 insert (~ 2420 base pairs)	≥ 99% sequence identity to human ACE2 (GenBank: GQ262784.1)	100% sequence identity to human ACE2 (GenBank: GQ262784.1)
Sequencing of modified pαH vector (~ 4600 base pairs)	C-terminal HRV3C protease cleavage site confirmed C-terminal <i>Strep-tag</i> [®] II confirmed C-terminal octa-histidine tag confirmed	C-terminal HRV3C protease cleavage site confirmed C-terminal <i>Strep-tag</i> [®] II confirmed C-terminal octa-histidine tag confirmed
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 (pre-vial) Digestion with <i>Cla</i> I	~ 7 kb	~ 7 kb (Figure 1) ^{1,3}
Concentration by PicoGreen[®] Measurement	≥ 2 µg/mL	0.8 µg in 20 µL per vial (38 µg/mL)
Amount per Vial	Report results	0.8 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	130 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.

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.

³*Cla*I has two restriction sites within NR-52565; however, one site is blocked by dam methylation, resulting in a single cut and linearization of the plasmid.

Figure 1: Agarose Gel of Undigested and Restriction Enzyme Digested NR-52565



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder

Lane 2: NR-52565 undigested

Lane 3: NR-52565 digested

/Heather Couch/

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Program Manager or designee, ATCC Federal Solutions

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