Certificate of Analysis for NR-52948

SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike-Pseudotyped Lentiviral Kit

Catalog No. NR-52948

Product Description:

The severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: NC_045512) spike-pseudotyped lentiviral kit (NR-52948) is designed to generate pseudotyped lentiviral particles with the spike (S) glycoprotein gene, as well as luciferase (Luc2) and green fluorescent protein (GFP).

The deposited plasmids were 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 vialed in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Table 1: Lentiviral Kit Components

COMPONENT NUMBER	DESCRIPTION	LOT NUMBER	DATE OF MANUFACTURE
NR-52514	Viral entry protein encoding for spike glycoprotein	70035472	29APR2020
NR-52516	Lentiviral Backbone encoding for Luc2 and ZsGreen	70035474	29APR2020
NR-52517	Helper plasmid encoding for Gag and Pol	70035478	29APR2020
NR-52518	Helper plasmid encoding for Tat1b	70035480	29APR2020
NR-52519	Helper plasmid encoding for Rev1b	70035482	29APR2020

Table 2: Viral Entry Protein (NR-52514)

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 8380 base pairs	8386 base pairs[1]
Genotypic Analysis Sequencing of S glycoprotein insert (~ 3820 base pairs)	≥ 99% sequence identity to depositor's sequence	100% sequence identity to depositor's sequence[2]
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene *bla*)[3]	*bla* sequence present	*bla* sequence present
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
OD_{260}/OD_{280} Ratio (pre-vial)	1.7 to 2.1	2.0
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 *E. coli*	≥ 50 colonies per ng	129 colonies per ng

[1]The sequence was assembled pre-vial using the depositor's predicted sequence as the reference sequence.
[2]The NR-52514 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).
[3]The 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.

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Table 3: Lentiviral Backbone (NR-52516)

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	~ 9370 base pairs	9369 base pairs[1]
Genotypic Analysis Sequencing of Luc2 gene (~ 1650 base pairs) Sequencing of ZsGreen1 gene (~ 700 base pairs)	 ≥ 99% sequence identity to depositor's sequence ≥ 99% sequence identity to depositor's sequence	 100% sequence identity to depositor's sequence 100% sequence identity to depositor's sequence
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene *bla*)[2]	 *bla* sequence present	 *bla* sequence present
Concentration by PicoGreen® Measurement	≥ 2 µg/mL	0.2 µg in 20 µL per vial (11 µg/mL)
Amount per Vial	Report results	0.2 µg per vial
OD_{260}/OD_{280} Ratio (pre-vial)	1.7 to 2.1	1.8
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 *E. coli*	 ≥ 50 colonies per ng	 > 500 colonies per ng

[1]The sequence was assembled pre-vial using the depositor's predicted sequence as the reference sequence.
[2]The 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.

Table 4: Helper plasmids (NR-52517 to NR-52519)

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	Report results	Consistent with depositor reported size[1]
Genotypic Analysis Sequencing of insertion	 ≥ 99% sequence identity to depositor's sequence	 100% sequence identity to depositor's sequence
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene *bla*)[2] Neomycin [NR-52519, encoded by aminoglycoside 3'-phosphotransferase gene aph(3')-II]	 *bla* sequence present aph(3')-II sequence present	 *bla* sequence present aph(3')-II sequence present
Concentration by PicoGreen® Measurement NR-52517 NR-52518 NR-52519	 ≥ 2 µg/mL ≥ 2 µg/mL ≥ 2 µg/mL	 0.2 µg in 20 µL per vial (12 µg/mL) 0.8 µg in 50 µL per vial (15 µg/mL) 0.9 µg in 70 µL per vial (13 µg/mL)
Amount per Vial NR-52517 NR-52518 NR-52519	 Report results Report results Report results	 0.2 µg 0.8 µg 0.9 µg
OD_{260}/OD_{280} Ratio (pre-vial)	1.7 to 2.1	1.7 to 2.1
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 *E. coli*	 ≥ 50 colonies per ng	 > 500 colonies per ng

[1]The sequence was assembled pre-vial using the depositor's predicted sequence as the reference sequence.
[2]The 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.

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/Heather Couch/
Heather Couch 29 MAY 2020
Program Manager or designee, ATCC Federal Solutions

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