Certificate of Analysis for HRP-20083

Human Immunodeficiency Virus Type 1 (HIV-1) Molecular Clone NL4-BAL-CO-nLuc

Catalog No. HRP-20083

This reagent is the tangible property of the U.S. Government.

Product Description:

HRP-20083 is a replication-competent, CCR5-tropic human immunodeficiency virus type 1 (HIV-1) reporter construct designed to encode a bioluminescent nanoluciferase (nLuc) protein upstream of the encephalomyocarditis virus internal ribosome entry site (IRES), 6ATRi, to allow expression of Nef. The plasmid encodes full-length, replication-competent HIV-1 in a pUC18 backbone. The reporter gene was codon optimized to remove cytosine/guanine (CG) dinucleotides, giving improved replication *in vitro* and reporter expression *in vivo* and *ex vivo*. The beta-lactamase gene, *bla*, provides transformant selection through ampicillin resistance in *Escherichia coli (E. coli)*. The resulting size of the plasmid is reported to be approximately 15000 base pairs. The deposited plasmid was diluted and vialed in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

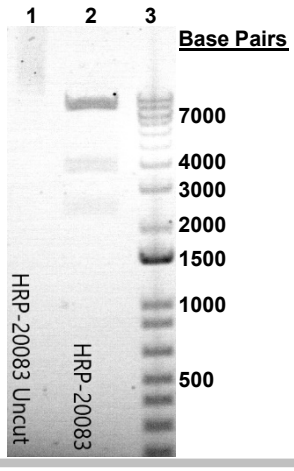
Lot: 70048377 **Preservation Date: 17DEC2021**

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	Report results	~ 13385 base pairs[1]
Genotypic Analysis Sequencing of pUC18 vector (~ 2060 base pairs) Sequencing of nLuc and 6ATRi IRES region (~ 990 base pairs)	 ≥ 99% sequence identity to predicted sequence ≥ 99% sequence identity to depositor's sequence	 99.9% sequence identity to predicted sequence 100% sequence identity to depositor's sequence
Antibiotic Resistance Ampicillin (encoded by beta-lactamase gene *bla*)[2]	 *bla* sequence present	 *bla* sequence present
Agarose Gel Electrophoresis		
Digestion with *Apa*I and *Sal*I	~ 10 kb and ~ 4 kb	~ 10 kb and ~ 4 kb (Figure 1)
Concentration by Qubit Fluorometer®	≥ 2 µg per mL	0.8 µg in 100 µL per vial (8.5 µg per mL)
Amount per Vial	Report results	0.8 µg per vial
OD_{260}/OD_{280} Ratio	1.7 to 2.1	1.9
Effective Bacterial Transformation Invitrogen™ One Shot™ TOP10 *E. coli*	 ≥ 50 colonies per ng	 206 colonies per ng

[1]The sequence was assembled pre-vial using the predicted sequence as the reference sequence. The complete plasmid sequence and insert map are provided on the HIV Reagent Program webpage.

[2]The 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 Undigested and Restriction Enzyme Digested HRP-20083



Lane 1: HRP-20083 undigested
Lane 2: HRP-20083 digested
Lane 3: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder

NIH HIV Reagent Program
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