



Certificate of Analysis for HRP-20084

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

Catalog No. HRP-20084

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

Product Description:

HRP-20084 is a replication-competent, CCR5-tropic human immunodeficiency virus type 1 (HIV-1) reporter construct designed to encode a near-infrared fluorescent protein (iRFP) 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 vialled in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Lot: 70048379

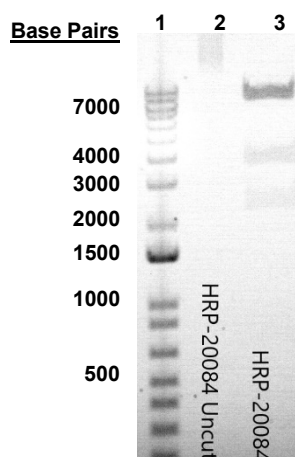
Preservation Date: 17DEC2021

TEST	SPECIFICATIONS	RESULTS
Next-Generation DNA Sequencing	Report results	~ 13385 base pairs ¹
Genotypic Analysis		
Sequencing of pUC18 vector (~ 2060 base pairs)	≥ 99% sequence identity to predicted sequence	99.9% sequence identity to predicted sequence
Sequencing of iRFP and 6ATRi IRES region (~ 1410 base pairs)	≥ 99% sequence identity to depositor's sequence	100% sequence identity to depositor's sequence
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>Apal</i> and <i>Sall</i>	~ 10 kb and ~ 4 kb	~ 10 kb and ~ 4 kb (Figure 1)
Concentration by Qubit Fluorometer®	≥ 2 µg per mL	0.9 µg in 100 µL per vial (9 µg per mL)
Amount per Vial	Report results	0.9 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	206 colonies per ng

¹The 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.

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 Undigested and Restriction Enzyme Digested HRP-20084



Lane 1: Invitrogen™ TrackIt™ 1 Kb Plus DNA Ladder
 Lane 2: HRP-20084 undigested
 Lane 3: HRP-20084 digested



**HIV REAGENT
PROGRAM**

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/Ken Crawford/

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13 JAN 2022

ATCC[®], on behalf of the NIH HIV Reagent Program, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC[®]'s knowledge.

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