Certificate of Analysis

pNLCoI4[*luc2*-P2A-*NlucP*/PGK/Hygro] Vector:

Part No.	Size
N149A	20µg

Part# 9PIN149
Revised 9/16

Description: The pNLCoI4[*luc2*-P2A-*NlucP*/PGK/Hygro] Vector[a,b] is a second generation coincidence reporter vector (CoI; 1) that allows expression of both firefly luciferase (Fluc, *luc2* gene) from *Photinus pyralis* and NanoLuc® Luciferase fused to a PEST destabilization domain (NanoLuc®-PEST, *NlucP* gene) from the same mRNA transcript. The stoichiometric expression of both luciferases is achieved by use of the P2A sequence from porcine teschovirus-1, which promotes a ribosomal skip and expression of the two unfused enzymes with different compound interaction profiles. When used in high-throughput screening, true hits will show a similar response from both reporters, reducing the number of false hits and the workload associated with follow-up screens.

The *luc2* and *NlucP* genes are codon optimized for mammalian expression and all pNL vectors have minimal consensus transcription factor-binding sites to reduce anomalous expression. The pNLCoI4[*luc2*-P2A-*NlucP*/PGK/Hygro] Vector contains a PGK promoter for moderate-level expression of both reporters, allowing its use as a constitutive expression control.

The pNLCoI4[*luc2*-P2A-*NlucP*/PGK/Hygro] Vector contains the following key features:

- Firefly luciferase reporter gene (*luc2*).
- P2A peptide sequence, promotes a ribosomal skip resulting in the stoichiometric expression of two unfused reporter proteins from the same mRNA transcript.
- The NanoLuc® Luciferase reporter gene fused to the PEST destabilization domain (*NlucP*). PEST-mediated turnover by the proteasome provides a reporter with a very short intracellular protein half-life, providing a reporter protein that responds more quickly and typically with greater signal-to-background compared to other reporters. NanoLuc®-PEST is also substantially brighter than *Renilla* luciferase, providing a dramatic increase in sensitivity for the second generation coincidence reporter system.
- Ampicillin resistance gene for selection in bacteria.
- Hygromycin resistance gene for selection in mammalian cells.
- Human phosphoglycerate kinase (PGK) promoter, a nonviral, universal promoter that provides moderate levels of expression in mammalian cells.

Concentration: 1µg/µl.

GenBank® Accession Number: KM376434.

Storage Buffer: The pNLCoI4[*luc2*-P2A-*NlucP*/PGK/Hygro] Vector is supplied in 10mM Tris-HCl (pH 7.4), 1mM EDTA.

Storage Conditions: See the product information label for storage recommendations and expiration date.

Quality Control Assays

Contaminant Assays

Contaminating Nucleic Acids: RNA, single-stranded DNA and chromosomal DNA are not evident in specified quantities of the vector as determined by agarose gel electrophoresis.

Endotoxin Concentration: Endotoxin Units (EU) are obtained using *Limulus amoebocyte* lysate testing. The specification is <100EU/mg of plasmid DNA.

Nuclease Assay: Following incubation of 1µg of the vector in restriction enzyme buffer at 37°C for 16–24 hours, no evidence of nuclease activity is detected by agarose gel electrophoresis.

Physical Purity: $A_{260}/A_{280} \geq 1.80$, $A_{260}/A_{250} \geq 1.05$ at pH 7.4.

Functional Assays

Identity Assay: The vector was sequenced completely and has 100% identity with the published sequence available at: www.promega.com/vectors

Restriction Digestion: The functional purity of vector DNA is verified by successful digestion with restriction enzymes at the optimal temperature for one hour. Samples are examined by agarose gel electrophoresis to compare cut and uncut vector DNA with marker DNA.

Reference

1. Cheng, K.C. and Inglese, J. (2012) A coincidence reporter-gene system for high-throughput screening. *Nat. Methods*, **9**, 937.

Signed by:

R. Wheeler, Quality Assurance

AF9PIN149 0916N149

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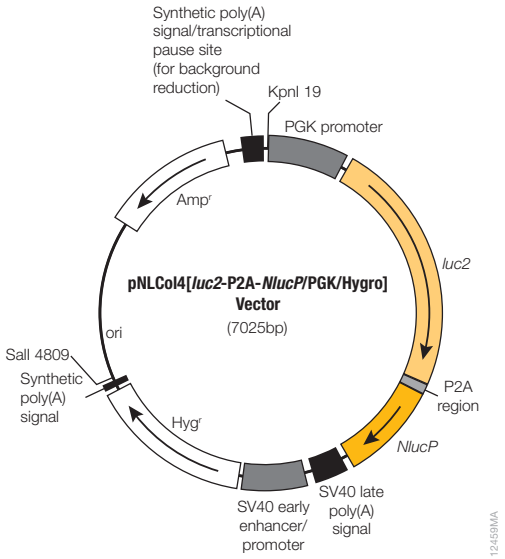
pNLCol4[*luc2*-P2A-*NlucP*/PGK/Hygro] Vector Features and Map

The following features are present in the vector based on nucleotide sequence.

PGK promoter	27–541
luc2 reporter gene	577–2226
P2A region	2227–2292
NlucP reporter gene	2293–2931
SV40 late poly(A) region	2971–3192
SV40 early enhancer/promoter	3240–3658
Hygromycin resistance coding region	3683–4720
Synthetic poly(A) sequence	4744–4792
Reporter Vector primer 4 (RVprimer4) binding region	4859–4878
*Col*EI-derived plasmid replication origin	5116
Synthetic β-lactamase (Amp^r) coding region	5907–6767
Synthetic poly(A) signal/transcriptional pause region	6872–7025
Reporter Vector primer 3 (RVprimer3) binding region	6974–6993



Sequence information and vector maps are available online: **www.promega.com/vectors**

For information on the Nano-Glo® Dual-Luciferase® Assay System see Technical Manual #TM426, available online: **www.promega.com/protocols**

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