Certificate of Analysis

Part# 9PIN141

Revised 9/16

pNL3.2.CMV Vector:

Part No.	Size
N141A	20µg

Description: The pNL3.2.CMV Vector[a,b] is designed for constitutive expression of the NanoLuc® reporter fused to a PEST destabilization domain (*NlucP*). The NlucP fusion protein naturally accumulates at low intracellular levels due to constitutive proteosomal degradation, thus serving as a negative control for experiments configured to measure regulated changes in NanoLuc® luciferase expression levels. This vector also encodes a hygromycin resistance gene for selection in mammalian cells and an ampicillin resistance gene for selection in bacteria.

The pNL3.2.CMV Vector contains the following features:

- A **CMV promoter** for robust expression in mammalian cells
- The **NanoLuc® reporter gene**, for high sensitivity detection of the protein of interest.
- **PEST** sequence for maintaining low intracellular NanoLuc® luciferase concentration.
- **Ampicillin resistance** gene for selection in bacteria.
- **Hygromycin resistance** gene for selection in mammalian cells.

Concentration: 1µg/µl.

GenBank® Accession Number: KF853603.

Storage Buffer: The pNL3.2.CMV Vector is supplied in 10mM Tris-HCl, 1mM EDTA (pH 7.4).

Storage Conditions: See the Product Information Label for storage recommendations and expiration date. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. These fluctuations can greatly alter product stability. See label for expiration date.

Usage Note: Concentration gradients may form in frozen products and should be dispersed upon thawing. Mix well prior to use.

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: $A_{260}/A_{280} \geq 1.80$, $A_{260}/A_{250} \geq 1.05$.

Functional Assays

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

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

Signed by:

R. Wheeler, Quality Assurance

AF9PIN141 0916N141

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Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on

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Usage Information

pNL3.2.CMV Vector Features

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

CMV immediate early enhancer/promoter	14–755
NanoLuc®-PEST protein coding region	859–1497
SV40 late poly(A) region	1537–1758
SV40 early enhancer/promoter	1806–2224
Synthetic hygromycin (Hyg^r) coding region	2249–3286
Beta-lactamase (Amp^r) coding region	4473–5333
ColE1-derived plasmid replication origin	3682–3718
Synthetic poly(A) signal	5438–5486

Related Products

Product	Size	Cat.#
Nano-Glo® Luciferase Assay	10ml	N1110
	100ml	N1120
	10 × 10ml	N1130
	10 × 100ml	N1150
FuGENE® HD Transfection Reagent	1ml	E2311
	5 × 1ml	E2312

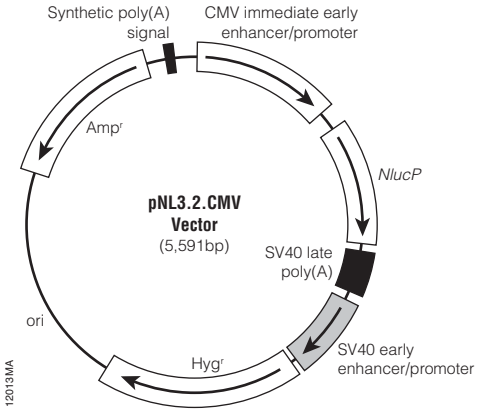

Figure 1. pNL3.2.CMV Vector circle map.

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[b]U.S. Pat. No. 8,557,970 and other patents pending.

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