Certificate of Analysis

pFN31K *Nluc* CMV-neo Flexi® Vector:

Part# 9PIN132

Printed 11/13

Part No.	Size
N132A	20µg

Description: The pFN31K *Nluc* CMV-neo Flexi® Vector[a,b] is configured to to facilitate simple, efficient transfer of the gene of interest into a vector designed for genetic attachment of NanoLuc® luciferase to the N-terminus of the protein of interest. This vector provides constitutive protein expression in mammalian cells using the human cytomegalovirus (CMV) immediate early enhancer/promoter. The vector can be used for both stable and transient gene expression. The vector backbone contains a neomycin phosphotransferase gene to allow selection in *E. coli* with kanamycin or in mammalian cell lines with neomycin.

The pFN31K *Nluc* CMV-neo Flexi® Vector contains the following features:

- A **CMV immediate early enhancer/promoter** for constitutive expression in mammalian cells.
- A **T7 RNA polymerase promoter** for cell-free expression of NanoLuc® fusion proteins.
- The **NanoLuc® gene** for high-sensitivity detection of NanoLuc® fusion proteins expressed in mammalian cells.
- The **lethal barnase gene** for positive selection of the insert. **Note:** The pFN31K *Nluc* CMV-neo Flexi® Vector can be propagated only in *E. coli* once the barnase gene is replaced with the protein-coding sequence of interest.
- A **neomycin/kanamycin-**resistance gene for selection in bacterial or mammalian cells.
- Unique **SgfI** and **PmeI** sites, which allow easy insertion of the sequence of interest from PCR product or compatible Flexi® vectors. When transferred in the proper reading frame, these sites create a readthrough sequence that can be joined to a protein-coding region flanked by SgfI and PmeI sites. In-frame transfer results in gene encoding a NanoLuc® fusion to the N-terminus of the protein of interest. Once inserted in this vector, the sequence is available for transfer to other Flexi® Vectors. For more information, see the *Flexi® Vector Systems Technical Manual* #TM254, available online at: **www.promega.com/protocols/**

Concentration: 100ng/µl.

GenBank® Accession Number: KF793053 .

Storage Buffer: The pFN31K *Nluc* CMV-neo Flexi® Vector is supplied in 10mM Tris-HCl, 1mM EDTA (pH 8.0).

Storage Conditions: See 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.

Usage Notes:

1. This vector was designed to be used with the Flexi® Vector System, a directional cloning method to shuttle protein-coding sequences between compatible vectors. To prepare the NanoLuc® fusion protein, the protein coding region is cloned into the pFN31K *Nluc* CMV-neo Flexi® Vector using the Flexi® System, Entry/Transfer (Cat.# C8640). For more information, see the *Flexi® Vector Systems Technical Manual* #TM254.

Quality Control Assays

Contaminant Assays

Contaminating Nucleic Acids: RNA, single-stranded DNA and chromosomal DNA are not evident in an overload sample of this vector as determined by agarose gel electrophoresis.

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 Digests: Vector DNA is analyzed for the presence of certain restriction enzyme sites by incubation with a variety of restriction enzymes at the specified digestion temperature for 1 hour. Samples are examined by agarose gel electrophoresis, comparing cut and uncut vector DNA with marker DNA.

Signed by: J. Stevens

J. Stevens, Quality Assurance

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Promega

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Usage Information

pFN31K *Nluc* CMV-neo Flexi® Vector Features

The following features are present based on nucleotide sequence.

CMV immediate early enhancer/promoter	1–742
Chimeric intron	857–989
T7 RNA polymerase promoter (–17 to +3)	1033–1052
NanoLuc® protein coding region	1065–1577
Linker	1578–1589
SgfI	1590–1597
Barnase coding region	1621–1956
PmeI	1958–1965
SV40 late poly(A) region	2117–2338
SV40 early enhancer/promoter	2437–2849
EM7 bacterial promoter	2863–2929
Neomycin phosphotransferase coding region	2943–3737
Synthetic poly(A) signal	3801–3849
ColE1-derived plasmid replication origin	4085–4121

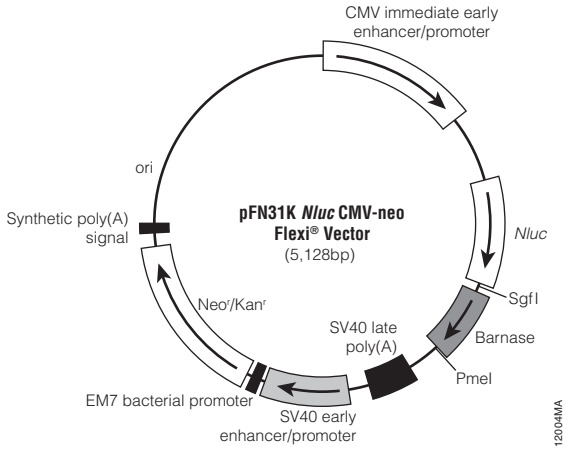

Figure 1. pFN31K *Nluc* CMV-neo Flexi® Vector circle map.

Related Products

Product	Size	Cat.#
Flexi® System, Entry/Transfer	5 entry and 20 transfer reactions	C8640
Flexi® System, Transfer	100 transfer reactions	C8820
Carboxy Flexi® System, Transfer	50 transfer reactions	C9320
10X Flexi® Enzyme Blend (SgfI & PmeI)	25µl	R1851
	100µl	R1852
Nano-Glo® Luciferase Assay	10ml	N1110
	100ml	N1120
	10 × 10ml	N1130
	10 × 100ml	N1150
FuGENE® HD Transfection Reagent	1ml	E2311
	5 × 1ml	E2312

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