Certificate of Analysis

pFC32K *Nluc* CMV-neo Flexi® Vector:

Part# 9PIN134

11/13

Part No.	Size
N134A	20µg

Description: The pFC32K *Nluc* CMV-neo Flexi® Vector[a,b] is configured to facilitate simple, efficient transfer of the gene of interest into a vector designed for genetic attachment of NanoLuc® luciferase to the C-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 pFC32K *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® luciferase**, for high sensitivity detection of NanoLuc® fusion proteins expressed in mammalian cells.
- The **lethal barnase gene** for positive selection of the insert. **Note: T**he pFC32K *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 **kanamycin/neomycin phosphotransferase gene** for selection of the plasmid in bacteria and mammalian cells.
- Unique **SgfI and EcoICRI 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 EcoICRI sites. In-frame transfer results in gene encoding a *Nluc* fusion to the C-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: KF811456.

Storage Buffer: The pFC32K *Nluc* CMV-neo Flexi® Vector is supplied in 10mM Tris-HCl, 1mM EDTA (pH 8.0).

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

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 pFC32K *Nluc* CMV-neo Flexi® Vector using the Flexi® System, Entry/Transfer (Cat.# C8640). For more information, see the *Flexi® Vector Systems Technical Manual* #TM254.
2. 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.

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 Digestion: 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: J. Stevens

J. Stevens, Quality Assurance

AF9PI N134 1113N134

Promega

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Usage Information

pFC32K *Nluc* CMV-neo Flexi® Vector Features

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

CMV immediate early enhancer/promoter	1–742
Chimeric intron	857–989
T7 RNA polymerase promoter (–17 to +3)	1033–1052
SgfI	1056–1063
Barnase coding region	1087–1422
EcoICRI	1442–1447
Linker	1447–1461
NanoLuc® protein coding region	1462–1971
SV40 late poly(A) region	2108–2329
SV40 early enhancer/promoter	2428–2840
EM7 bacterial promoter	2854–2920
Neomycin phosphotransferase coding region	2934–3728
Synthetic poly(A) signal	3792–3840
ColE1-derived plasmid replication origin	4076–4112

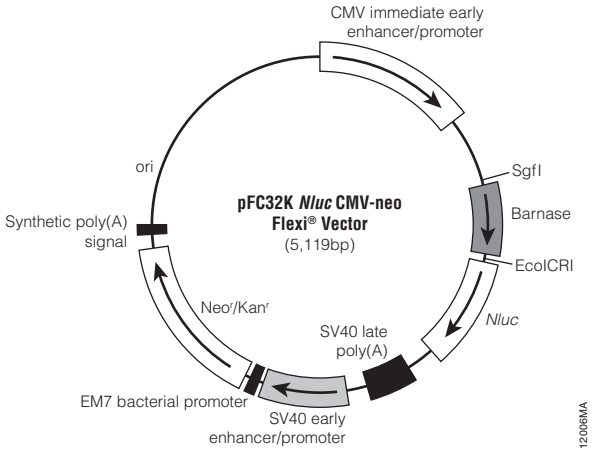

Figure 1. pFC32K *Nluc* CMV-neo Flexi® Vector circle map.

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
Carboxy Flexi® Enzyme Blend (SgfI & EcoICRI)	50µl	R1901
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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