

Certificate of Analysis

pFN38K HiBiT CMV-neo Flexi® Vector:

Part No. Size
N240A 20µg

Part# 9PIN240

Printed 8/17



Instructions for use of this product can be found in the *Nano-Glo® HiBiT Lytic Detection System Technical Manual #TM516* and *Nano-Glo® HiBiT Extracellular Detection System Technical Manual #TM523*, available online at: www.promega.com/protocols

Description: The pFN38K HiBiT 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 the HiBiT peptide tag to the N terminus of the protein of interest using the Flexi® Cloning System (Cat.# C8640). The vector can be used for both stable and transient gene expression and encodes kanamycin resistance for bacterial selection and neomycin resistance for mammalian selection.

The pFN38K HiBiT CMV-neo Flexi® Vector contains the following features:

- A **CMV immediate-early enhancer/promoter** for constitutive expression in mammalian cells.
- The **HiBiT peptide tag** for bioluminescent detection of the protein of interest.
- The **lethal barnase gene** for positive selection of the insert. **Note:** The pFN38K HiBiT CMV-neo Flexi® Vector can only be propagated in *E. coli* once the barnase gene is replaced with the protein-coding sequence of interest.
- A **kanamycin-resistance gene** for selection of the plasmid in bacteria and a **neomycin-resistance gene** for selection in mammalian cells.
- Unique **SgfI** and **PmeI** sites, which allow easy insertion of any protein-coding sequence flanked by SgfI and PmeI sites (e.g., from PCR products or N-terminal Flexi® Vectors). In-frame transfer results in a gene encoding a HiBiT 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: 1 µg/µl.

Storage Buffer: The pFN38K HiBiT CMV-neo Flexi® Vector is supplied in 10mM Tris-HCl, 1mM EDTA (pH 7.4).

Storage Conditions: Store at -30°C to -10°C.

Usage Notes:

- Expression of the HiBiT-tagged protein will only result when the proper reading frame is maintained between the HiBiT tag and the gene of interest.
- Avoid multiple freeze-thaw cycles.

Expiration Date: See product label for 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.

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

Functional Assays

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

Restriction Digestion: The functional purity of the vector DNA is verified by successful digestion with restriction enzymes at the optimal temperature for 1 hour. Samples are examined by agarose gel electrophoresis, comparing cut and uncut vector DNA with marker DNA.



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^(a)Patents Pending.

^(b)U.S. Pat. Nos. 8,293,503, 9,018,014, and 8,367,403, European Pat. No. 1685247 and other patents and patents pending.

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Signed by:

R. Wheeler, Quality Assurance

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pFN38K HiBiT CMV-neo Flexi® Vector Features and Circle Map

The following features are present in the pFN38K HiBiT CMV-neo Flexi® 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
HiBiT	1065–1100
SgfI site	1125–1132
Barnase coding region	1156–1491
PmeI site	1493–1500
SV40 late polyadenylation signal	1652–1873
SV40 enhancer and early promoter	1972–2390
SV40 enhancer	2045–2281 (Reverse)
SV40 Min Ori	2288–2353
EM7 bacterial promoter	2398–2464
Neo-Kan resistance	2478–3272
Synthetic polyadenylation signal sequence	3336–3384
Col/E1-derived plasmid origin of replication	3620–3656

Related Products

Product	Size	Cat. #
Nano-Glo® HiBiT Lytic Detection System	10ml	N3030
	100ml	N3040
	10 × 100ml	N3050
Nano-Glo® HiBiT Extracellular Detection System	10ml	N2420
	100ml	N2421
	10 × 100ml	N2422
Nano-Glo® HiBiT Blotting System	100ml	N2410

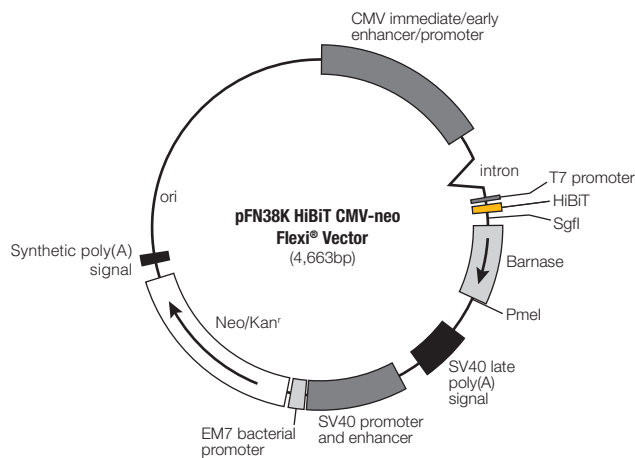


Figure 1. pFN38K HiBiT CMV-neo Flexi® Vector circle map and sequence reference points.