

Certificate of Analysis

pFC37K HiBiT CMV-neo Flexi® Vector:

Part No. Size
N239A 20µg

Part# 9PIN239

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 pFC37K 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 C 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 pFC37K 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 pFC37K 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 transfer of any protein-coding sequence flanked by SgfI and PmeI sites (e.g., PCR products or N-terminal Flexi® Vectors). In-frame transfer results in a gene encoding a HiBiT fusion to the C terminus of the protein of interest. For more information, see the *Flexi® Vector Systems Technical Manual #TM254*, available online at: www.promega.com/protocols/

Concentration: 1µg/µl.

Storage Buffer: The pFC37K 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.
- The insert should not encode a stop codon.
- The gene of interest should contain proper translation initiation sequences, including an N-terminal ATG codon or Kozak sequences.
- 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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pFC37K HiBiT CMV-neo Flexi[®] Vector Features and Circle Map

The following features are present in the pFC37K 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
SgfI site	1056–1063
Barnase coding region	1087–1422
EcoICRI site	1442–1447
HiBiT	1474–1506
SV40 late polyadenylation signal	1643–1864
SV40 enhancer and early promoter	1963–2381
SV40 enhancer	2036–2272 (Reverse)
SV40 Min Ori	2279–2344
EM7 bacterial promoter	2389–2455
Neo-Kan resistance	2469–3263
Synthetic polyadenylation signal sequence	3327–3375
ColE1-derived plasmid origin of replication	3611–3647

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

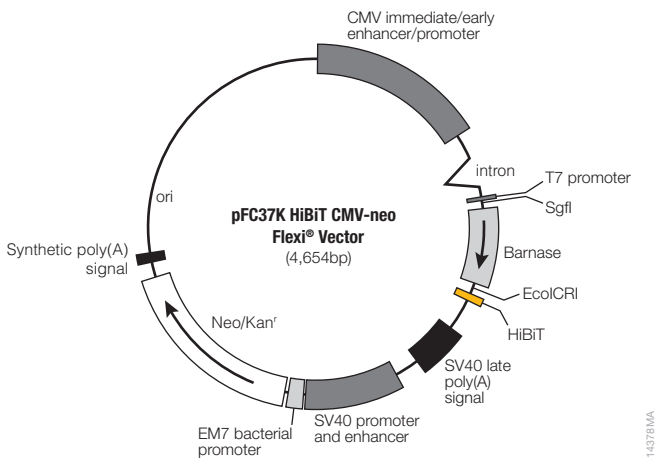


Figure 1. pFC37K HiBiT CMV-neo Flexi[®] Vector circle map and sequence reference points.