

## Certificate of Analysis

### pFN39K secHiBiT CMV-neo Flexi<sup>®</sup> Vector:

Part No.                      Size  
N241A                         20µg

Part# 9PIN241

Printed 8/17



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

**Description:** The pFN39K secHiBiT CMV-neo Flexi<sup>®</sup> Vector<sup>(a,b)</sup> 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 amino terminus of the mature form of transmembrane or secreted proteins using the Flexi<sup>®</sup> Cloning System (Cat.# C8640). The vector encodes the IL-6 secretion signal peptide N-terminal to the HiBiT tag for direct trafficking of HiBiT-tagged proteins to the plasma membrane of mammalian cells. 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 pFN39K secHiBiT CMV-neo Flexi<sup>®</sup> Vector contains the following features:

- A **CMV immediate-early enhancer/promoter** for constitutive expression in mammalian cells.
- A sequence encoding an **N-terminal IL-6 secretion sequence** for efficient cell-surface trafficking.
- The **HiBiT peptide tag** for bioluminescent detection of the protein of interest.
- The **lethal barnase gene** for positive selection of the insert. **Note:** The pFN39K secHiBiT CMV-neo Flexi<sup>®</sup> 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<sup>®</sup> Vectors). In-frame transfer results in a gene encoding an IL-6-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<sup>®</sup> Vectors. For more information, see the *Flexi<sup>®</sup> Vector Systems Technical Manual #TM254*, available online at: [www.promega.com/protocols/](http://www.promega.com/protocols/)

**Concentration:** 1 µg/µl.

**Storage Buffer:** The pFN39K secHiBiT CMV-neo Flexi<sup>®</sup> Vector is supplied in 10mM Tris-HCl, 1mM EDTA (pH 7.4).

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

#### Usage Notes:

- We recommend removing naturally encoded secretion signal sequences from the gene of interest for efficient cell-surface expression of the HiBiT-tagged protein.
- 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](http://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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**Promega**

#### Promega Corporation

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<sup>(a)</sup>Patents Pending.

<sup>(b)</sup>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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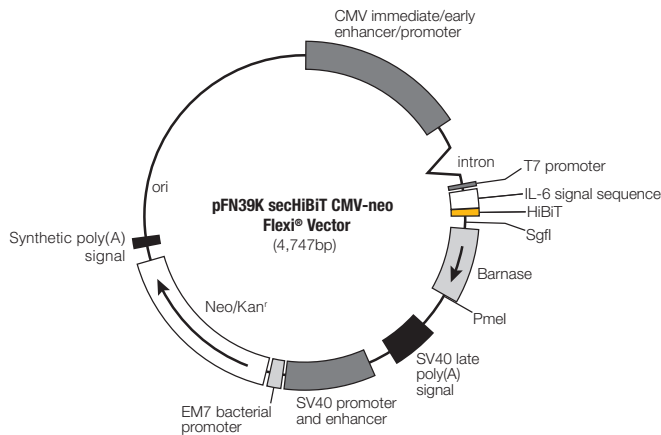
## pFN39K secHiBiT CMV-neo Flexi<sup>®</sup> Vector Features and Circle Map

The following features are present in the pFN39K secHiBiT CMV-neo Flexi<sup>®</sup> 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           |
| IL-6 signal sequence                         | 1065–1151           |
| HiBiT  | 1152–1184           |
| SgfI site                                    | 1209–1216           |
| Barnase coding region                        | 1240–1575           |
| PmeI site                                    | 1577–1584           |
| SV40 late polyadenylation signal             | 1736–1957           |
| SV40 enhancer and early promoter             | 2056–2474           |
| SV40 enhancer                                | 2129–2365 (Reverse) |
| SV40 Min Ori                                 | 2372–2437           |
| EM7 bacterial promoter                       | 2482–2548           |
| Neo-Kan resistance                           | 2562–3356           |
| Synthetic polyadenylation signal sequence    | 3420–3468           |
| Col/E1-derived plasmid origin of replication | 3704–3740           |

## Related Products

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



**Figure 1.** pFN39K secHiBiT CMV-neo Flexi<sup>®</sup> Vector circle map and sequence reference points.