Certificate of Analysis

pBiT3.1-secN [CMV/HiBiT/Blast] Vector:

Part No.	Size
N238A	20µg

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 pBiT3.1-secN [CMV/HiBiT/Blast] Vector[a] is configured to append the 11 amino acid HiBiT peptide tag to the amino terminus of the mature form of transmembrane or secreted proteins. 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 contains a multiple cloning region to generate an in-frame HiBiT fusion protein. The vector also encodes a blasticidin-resistance gene for selection in mammalian cells and a kanamycin-resistance gene for selection in bacteria.

The pBiT3.1-secN [CMV/HiBiT/Blast] 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.
- A **multiple cloning region** containing unique restriction sites to facilitate gene insertion into the vector.
- A sequence encoding a flexible **linker** between the protein of interest and the HiBiT tag.
- A **kanamycin-resistance gene** for selection of the plasmid in bacteria and a **blasticidin-resistance gene** for selection in mammalian cells.

Concentration: 1µg/µl.

Storage Buffer: The pBiT3.1-secN [CMV/HiBiT/Blast] 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 flexible linker will be variable in length depending on the restriction enzyme used.
- The insert should also contain a stop codon at the 3´ end for termination of the translation.
- 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.

Signed by:

R. Wheeler, Quality Assurance

Part# 9PIN238

Printed 8/17

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Promega

Promega Corporation

2800 Woods Hollow Road	
Madison, WI 53711-5399	USA
Telephone	608-274-4330
Toll Free	800-356-9526
Fax	608-277-2516
Internet	www.promega.com

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[a]Patents Pending.

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Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

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Usage Information

pBiT3.1-secN [CMV/HiBiT/Blast] Vector Features and Circle Map

The following features are present in the pBiT3.1-secN [CMV/HiBiT/Blast] Vector based on nucleotide sequence.

CMV promoter	276–866
Chimeric intron	981–1113
T7 RNA polymerase promoter (–17 to +3)	1157–1176
IL-6 signal sequence	1190–1276
HiBiT	1277–1309
SV40 late polyadenylation signal	1464–1685
EM7 bacterial promoter	1751–1817
Neo-Kan resistance	1831–2625
*Col*E1-derived plasmid origin of replication	2780–2816
Synthetic polyadenylation signal sequence	3497–3545 (Reverse)
Blasticidin resistance (Blast^r) coding region	3569–3967 (Reverse)
SV40 Min Ori	4029–4094 (Reverse)
SV40 Enhancer	4101–4337

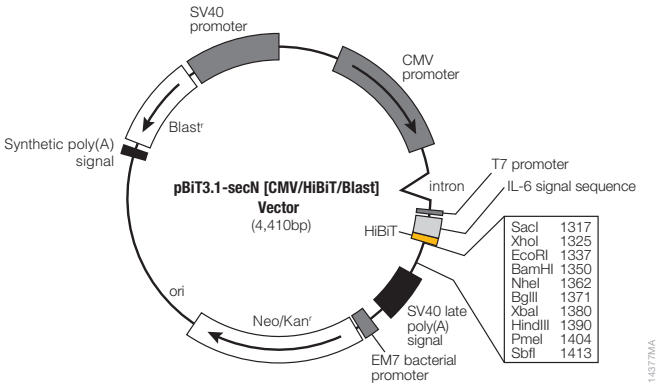

Figure 1. pBiT3.1-secN [CMV/HiBiT/Blast] Vector circle map and sequence reference points.

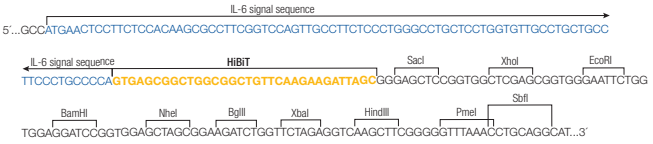

Figure 2. pBiT3.1-secN [CMV/HiBiT/Blast] Vector multiple cloning region sequence and unique restriction sites.

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

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