Certificate of Analysis

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

Part No.	Size
N237A	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-C [CMV/HiBiT/Blast] Vector[a] is configured to append the 11 amino acid HiBiT peptide tag to the carboxy terminus of the target protein. The vector contains a multiple cloning region to generate an in-frame HiBiT fusion protein. The vector can be used for both stable and transient gene expression and encodes kanamycin resistance for bacterial selection and blasticidin resistance for mammalian selection.

The pBiT3.1-C [CMV/HiBiT/Blast] 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.
- 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-C [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 not encode a stop codon.
- The gene of interest should contain proper translation initiation sequences, including an N-terminal ATG codon or Kozak sequence.
- 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# 9PIN237

Printed 8/17

AF9PIN237 0817N237

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

[a]Patents Pending.

Part# 9PIN237
Printed in USA 8/17

Usage Information

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

The following features are present in the pBiT3.1-C [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
HiBiT	1262–1294
SV40 late polyadenylation signal	1377–1598
EM7 bacterial promoter	1664–1730
Neo-Kan resistance	1744–2538
*Col*E1-derived plasmid origin of replication	2693–2729
Synthetic polyadenylation signal sequence	3410–3458 (Reverse)
Blasticidin resistance (Blast^r) coding region	3482–3880 (Reverse)
SV40 Min Ori	3942–4007 (Reverse)
SV40 Enhancer	4014–4250

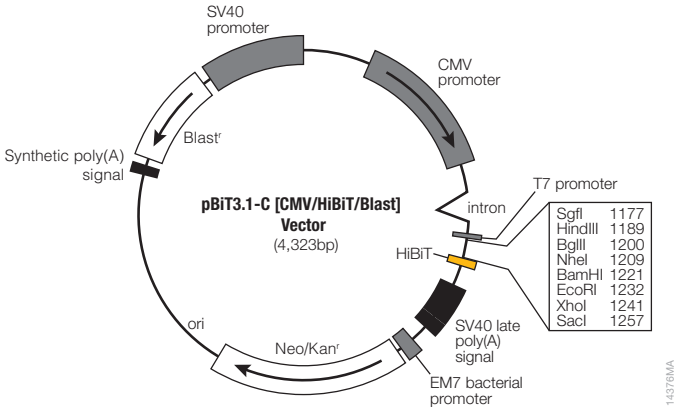

Figure 1. pBiT3.1-C [CMV/HiBiT/Blast] Vector circle map and sequence reference points.

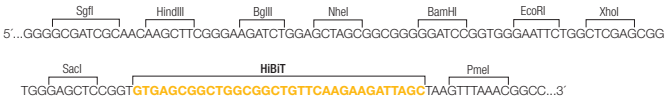

Figure 2. pBiT3.1-C [CMV/HiBiT/Blast] Vector multiple cloning region sequence and unique restriction sites.

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

Part# 9PIN237
Printed in USA 8/17