

Certificate of Analysis

Autophagy LC3 HiBiT Reporter Vector:

Part No. Size
GA255A 20µg

Part# 9PIGA255

Printed 1/18



Instructions for use of this product can be found in the *Autophagy LC3 HiBiT Reporter Assay System Technical Manual #TM535* and *Nano-Glo[®] HiBiT Lytic Detection System Technical Manual #TM516*, available online at:
www.promega.com/protocols

Description: The Autophagy LC3 HiBiT Reporter Vector^(a-e) is part of a bioluminescent, plate-based method for quantitative assessment of autophagy. This vector can be used to stably express the reporter in a chosen cell line.

The Autophagy LC3 HiBiT Reporter Vector contains the following features:

- An **HSV-TK promoter** with **PyF101 enhancer** for low-level, constitutive reporter expression in mammalian cells.
- The **HiBiT peptide tag** for bioluminescent detection of expressed reporter protein.
- A sequence encoding the **MAP1LC3B gene** with a **Spacer** coding sequence separating the gene from the HiBiT tag.
- A **kanamycin-resistance gene** for selection of the plasmid in bacterial cells and a **neomycin-resistance gene** for selection in mammalian cells.

Concentration: 1mg/ml.

Storage Buffer: The Autophagy LC3 HiBiT Reporter Vector is supplied in 10mM Tris-HCl, 1mM EDTA (pH 7.4).

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

Usage Note: Avoid multiple freeze-thaw cycles.

Expiration Date: See the product label for expiration date.



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Promega

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Quality Control Assays

This lot passes the following Quality Control specifications:

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

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^(d)U.S. Pat. No. 9,797,890 and other patents and patents pending.

^(e)U.S. Pat. Nos. 7,425,436, 7,935,803, 8,466,269, 8,742,086, 8,420,367 and 8,748,148 and other patents and patents pending.

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Autophagy LC3 HiBiT Reporter Vector Features and Circle Map

The following features are present in the vector based on nucleotide sequence.

PyF101 enhancer	44–239
HSV-TK promoter	241–993
HiBiT	1042–1077
Spacer	1078–2028
MAP1LC3B	2029–2403
SV40 late polyadenylation signal	2531–2752
SV40 enhancer and early promoter	2851–3269
EM7 bacterial promoter	3277–3343
Neo-Kan Resistance	3357–4151
Synthetic polyadenylation signal sequence	4215–4263
ColE1-derived plasmid replication origin	4499–4535
ApaLI site	4755–4760

Related Products

Product	Size	Cat. #
Nano-Glo® HiBiT Lytic Detection System	10ml	N3030
	100ml	N3040
	10 × 100ml	N3050
HEK293 Autophagy LC3 HiBiT Reporter Cell Line and Detection System	1 each	GA1040
U2OS Autophagy LC3 HiBiT Reporter Cell Line and Detection System	1 each	GA1050
CellTox™ Green Cytotoxicity Assay	200µl	G8731
	10ml	G8741
	50ml	G8742
	100ml	G8743

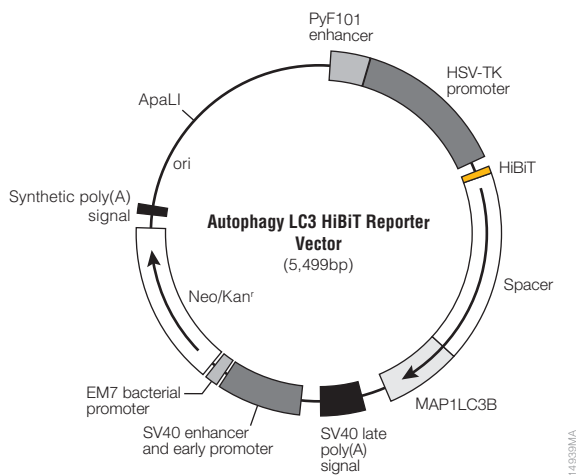


Figure 1. Autophagy LC3 HiBiT Reporter Vector circle map and sequence reference points.