Certificate of Analysis

Autophagy LC3 HiBiT Reporter Vector:

Part No.	Size
GA255A	20µg

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

Part# 9PIGA255

Printed 1/18

AF9PIGA2550118GA255

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 promotor** 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.

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

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

Part# 9PIGA255
Printed in USA 1/18.

[a]BY USE OF THIS PRODUCT, RESEARCHER AGREES TO BE BOUND BY THE TERMS OF THIS LIMITED USE STATEMENT. Researchers shall have no right to modify or otherwise create variations of the nucleotide sequences that encode the HiBiT and HaloTag® peptides. Researchers must: (1a) use Nano-Glo® HiBiT Lytic Detection System-branded luminescent assay reagents (LARs) for all determinations of luminescence activity of this product and its derivatives; or (1b) contact Promega to obtain a license for use of the product and its derivatives with LARs not manufactured by Promega. For uses of Nano-Glo®-branded LARs intended for energy transfer to acceptors other than a genetically encoded autofluorescent protein, researchers must: (2a) use NanoBRET™-branded energy acceptors for all determinations of energy transfer activity by this product and its derivatives; or (2b) contact Promega to obtain a license for use of the product and its derivatives for energy transfer assays to energy acceptors not manufactured by Promega. For uses of HaloTag® Technology in this product, researchers must: (1) use Promega HaloTag® ligands, which can be modified or linked to Promega or customer-supplied moieties, or (2) contact Promega to obtain a license if Promega HaloTag® ligands are not to be used. This product is for research use only; no other use of this product or derivatives is authorized. No transfer of this product is allowed. For a full copy of this label license, including the definition of "commercial use," go to: www.promega.com/LULL

[b]BY USE OF THIS PRODUCT, RESEARCHER AGREES TO BE BOUND BY THE TERMS OF THIS LIMITED USE LABEL LICENSE. Researcher shall have no right to modify or otherwise create variations of the product. Researcher must (a) use NanoBRET™-branded energy acceptors (e.g., BRET-optimized HaloTag® ligands) for all determinations of energy transfer activity by this product; or (b) contact Promega to obtain a license for use of this product for energy transfer assays to energy acceptors not manufactured by Promega. For any uses outside this label license, contact Promega for supply and licensing information. This product is for research use only; no transfer or commercial use is allowed. For a full copy of this label license, including the definition of "commercial use," go to: www.promega.com/LULL

[c]Licensed from Kazusa DNA Research Institute.

[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.

Usage Information

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

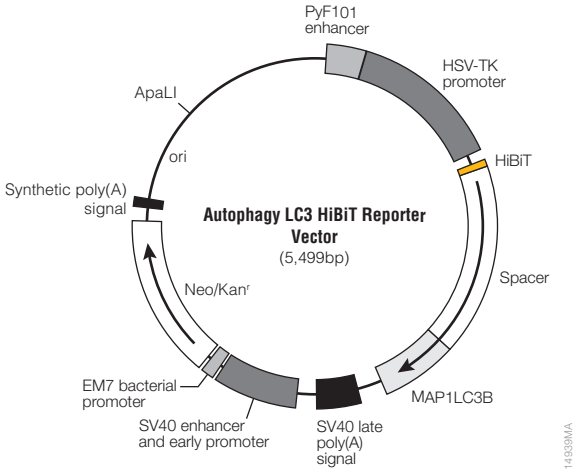

Figure 1. Autophagy LC3 HiBiT Reporter Vector circle map and sequence reference points.

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

Part# 9PIGA255
Printed in USA 1/18.