Review InvivoGen

Introducing Lucia: a new secreted luciferase

A NEW SECRETED LUCIFERASE OPTIMIZED TO ADVANCE CELL BASED REPORTER TECHNOLOGY

Luciferases encompass a wide range of enzymes used for bioluminescence, the emission of light produced by a living organism. Luciferases are highly prized bioindicators for life science research and drug discovery, owing to their remarkable sensitivity, lack of toxicity and wide dynamic range of quantitation.
Luciferases are used for many bioluminescence applications including gene reporter assays, whole-cell biosensor measurements, protein interaction studies using bioluminescence resonance energy transfer (BRET), drug discovery through high throughput screening and in vivo imaging.

The best studied luciferases, derived from the firefly and the sea pansy Renilla, are intracellular reporters and are associated with the need to lyse cells in order to measure bioluminescence.
One of the several advantages of secreted luciferases is the ease of detection directly from the cell culture medium, enabling kinetic studies from the same cells. Despite their many advantages, secreted luciferases are overshadowed by the traditional firefly and Renilla luciferases.

SECRETED LUCIFERASES

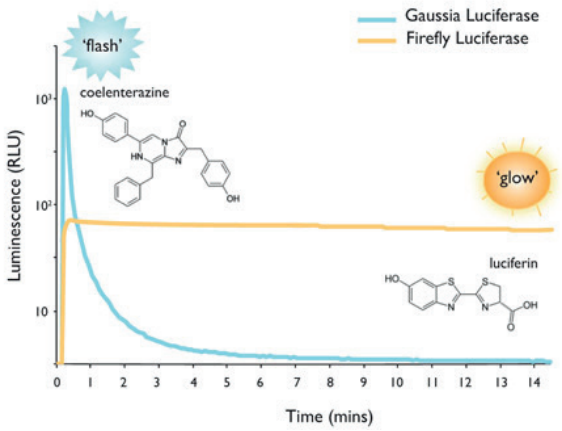
Naturally secreted forms of luciferases from marine bioluminescent organisms have been known for over 100
years, although their identification is relatively recent. The first secreted luciferase to be cloned was isolated from the marine ostracod crustacean, Vargula hilgendorfii, formerly known as Cypridina hilgendorfii, of the Cypridinidae family [1].
Later, secreted luciferases were isolated from luminous glands of marine copepod crustaceans Gaussia princeps [2], Metridia longa [3] and Metridia pacifica [4] of the Metridinidae family.
Earlier this year, newly identified secreted luciferases were cloned from copepods of Heterorhabdidae, Lucicutiidae and Augaptilidae families, isolated from Zooplankton collected off the deep sea of Japan [5].
The secreted copepod luciferases were functionally tested, and sequence alignment of the genes revealed a high degree of similarity in the primary structure. Copepod luciferases share two catalytic domains, D1 and D2, and an amino-terminal signal sequence.

Conveniently, when expressed in mammalian or insect cells, the native signal sequences of these luciferases are functionally active, mediating their export from within the cell to the surrounding culture medium. Bioluminescence assays are simply conducted using culture media, whereupon the activity of the secreted luciferases provides a readout of the biological signaling event under study. This aspect together with the strong intensity of bioluminescent signal generated, make secreted luciferases appealing for the design of novel reporter genes with enhanced properties [6-8]. Applications of secreted luciferases extend beyond their use as in vitro cell biological reporters, and can be adapted for real-time ex vivo monitoring of in vivo biological processes [9-11].

INVIVOGEN'S LUCIA LUCIFERASE

InvivoGen's Lucia luciferase is a completely novel secreted luciferase expressed by a synthetic gene designed on the naturally secreted luciferases from marine copepods. Lucia luciferase has been engineered for its superior properties compared to natural secreted luciferases.
The superior bioluminescence signal generated by Lucia luciferase is magnitudes stronger than the commonly used firefly and Renilla luciferases. The intense bioluminescence facilitates real-time measurements to detect very small amounts of the reporter in the cell culture medium and slight changes in the reporter concentration. Furthermore, the Lucia luciferase gene is codon optimized and free of CpG dinucleotides for prolonged mammalian cell expression.

A major challenge in the use of cell-based assays is establishing cell lines that reliably express a reporter gene without experiencing diminishing expression with increasing passages. Expression of the Lucia luciferase gene is designed for stable expression providing reliability and biological significance between experiments.



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LUCIFERASE (SPECIES)	SECRETED	SIZE (KDA)	EMISSION TYPE (T1/2)
Luciferin utilizing luciferases			
Photinus pyralis (firefly)	no	61	Glow
Vargula hilgendorfii	yes	62	Glow
Coelenterazine utlizing luciferases			
Lucia luciferase	yes	23	Extended flash (5 mins)
Renilla reniformis	no	36	Flash (30 sec)
Gaussia princeps	yes	20	Flash
Metridia longa	yes	24	Flash
Metridia pacifica	yes	20-23	Flash

LUCIFERASE DETECTION REAGENTS

The phenomenon of bioluminescence is the emission of visible light produced when luciferases catalyze the oxidation of specific substrates. Substrates of luciferases can be broadly classed into two groups; luciferins and coelenterazines.

Luciferases, such as firefly, that use luciferin or derivatives as substrates require ATP and Mg2+ as cofactors and display stable “glow” kinetics. The light emitted is generally in the green-yellow region of the visible light spectrum.

Conversely, luciferases using coelenterazine do not require ATP for activity and produce a rapid, often intense, “flash” light. Renilla luciferase along with the secreted copepod luciferases known to date display substrate specificity toward coelenterazine. The coelenterazine-utilizing luciferases emit visible blue light with a wavelength between 465-493 nm.

Coelenterazine, as a molecule, is unstable with respect to its auto-oxidation potential. Spontaneously, coelenterazine is oxidized to coelenteramide yielding the visible blue light and release of carbon dioxide. A number of variants and synthetic analogs have been used to improve stability by preventing autooxidation, in order to reduce background signal during assaying [12].

InvivoGen’s QUANTI-Luc™ is a one-step detection reagent paired for use with Lucia luciferase. QUANTI-Luc™ contains coelenterazine with stabilizing agents that limits substrate auto-oxidation and allows for the reconstituted reagent to be stored.
When used in combination with Lucia luciferase, the bioluminescent flash signal generated is longer-lasting, providing flexibility for taking readings.

InvivoGen offers reporter cell lines expressing the Lucia luciferase reporter gene, either alone or in combination with a secreted embryonic alkaline phosphatase (SEAP) reporter gene, allowing for concomitant monitoring of two distinct signaling pathways. Additionally, InvivoGen offers Lucia luciferase in a variety of mammalian expression plasmids, the recombinant protein and an anti-Lucia antibody.

For more information, please visit : www.invivogen.com

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REFERENCES

1. Thompson EM. et al., 1989. Cloning and expression of cDNA for the luciferase from the marine ostracod Vargula hilgendorfii. Proc Natl Acad Sci U S A 86: 6567-71.
2. Verhaegent M. & Christopoulos TK., 2002. Recombinant Gaussia luciferase. Overexpression, purification, and analytical application of a bioluminescent reporter for DNA hybridization. Anal Chem 74: 4378-85.
3. Markova SV. et al., 2004. Cloning and expression of cDNA for a luciferase from the marine copepod Metridia longa. A novel secreted bioluminescent reporter enzyme. J Biol Chem 279: 3212-17.
4. Takenaka Y. et al., 2008. Two forms of secreted and thermostable luciferases from the marine copepod crustacean, Metridia pacifica. Gene 425: 28-35.
5. Takenaka Y. et al., 2012. Evolution of bioluminescence in marine planktonic copepods. Mol Biol Evol 29(6):1669-81
6. Kim SB. et al., 2011. Superluminescent Variants of Marine Luciferases for Bioassays. Anal Chem. 83(22):8732-40.
7. Maguire CA. et al., 2009. Gaussia luciferase variant for high-throughput functional screening applications. Anal Chem 81: 7102-06.
8. Enjalbert B. et al., 2009. A multifunctional, synthetic Gaussia princeps luciferase reporter for live imaging of Candida albicans infections. Infect Immun 77: 4847-58.
9. Wurdinger T. et al. 2009. A secreted luciferase for ex vivo monitoring of in vivo processes. Nat Methods. 5(2): 171-173.
10. Tannous BA. & Teng J., 2011 Secreted blood reporters: Insights and applications. Biotechnol Adv 29: 997-1003.
11. Ray P. & Gambhir SS., 2007. Noninvasive imaging of molecular events with bioluminescent reporter genes in living subjects. Methods Mol Biol 411: 131-144.
12. Zhao H., 2004. Characterization of coelenterazine analogs for measurements of Renilla luciferase activity in live cells and living animals. Mol Imaging 3(1):43-54.

PRODUCTS - INVIVOGEN.COM

PRODUCT	DESCRIPTION
QUANTI-Luc™	Secreted luciferase detection medium
Streptavidin-Lucia	Bioluminescent conjugate of Streptavidin
Recombinant Lucia Luciferase Protein	Positive control for luciferase reporter assays
Lucia luciferase Gene	Lucia luciferase Reporter Gene in expression plasmid
Anti-Lucia-IgG	Lucia luciferase Neutralizing antibody - Monoclonal Mouse IgG1
Lucia luciferase Reporter Cell Lines	Reporter cell lines that feature the Lucia luciferase reporter gene
pNiFty3-Lucia	Signal Transduction Reporter Plasmids
pSELECT Lucia-Tag	Expression plasmid with a secreted luciferase (Lucia) Tag
pVITRO1-Lucia/SEAP	Dual reporter plasmids - EF-1α promoters - luciferase & SEAP
pVITRO2-Lucia/SEAP	Dual reporter plasmids - Ferritin promoters - Lucia & SEAP
pFUSE-Lucia-CHIg	Cloning plasmids with constant regions of the heavy (CH) chain and a secreted luciferase gene
pFUSE-Lucia-Fc	Production of Lucia luciferase-tagged Immunoadhesins

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