

Humanized *Gaussia* Luciferase

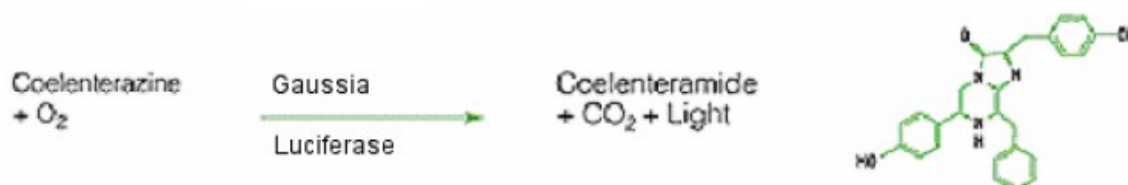
Gaussia luciferase, a novel reporter for gene expression, is the smallest and brightest known luciferase. Great for studying weak promoters or hard-to-transfect cells.

Product Information

Product name cat.number	Description
pGluc-Basic-1 vector <i>Gaussia</i> Luciferase, with secretion signal FP-BU2550, 25 µg	A promoterless vector with a MCS site upstream of the humanized <i>Gaussia</i> luciferase coding sequence (with secretion signal). This vector is designed for promoter analysis and will express secreted <i>Gaussia</i> luciferase. This avoids the lysis of the cells to assay the luciferase activity. The transfected cells can be reused for multiple sampling.
pCMV-Gluc-1 positive control, with secretion signal FP-BS8160, 25 µg	This positive control vector is very useful in evaluating the efficiency of transgene expression using <i>Gaussia</i> luciferase as a reporter. This vector has both Ampicillin resistance and Neomycin resistance. Therefore it can be easily propagated in <i>E. coli</i> and can be used to establish stable cell lines expressing <i>Gaussia</i> luciferase.
<i>Gaussia</i> Luciferase Assay kit FP-BY7160, 5 ml (100 tests) FP-BY7161, 50 ml (1000 tests)	2 components : predissolved coelenterazine (100X concentration) and an assay buffer with stabilizers.

Introduction

The marine luciferase, cloned from the copepod *Gaussia princeps*, catalyzes the oxidation of the small molecule coelenterazine to produce light. Unlike the firefly luciferase systems, these coelenterazine-utilizing luciferases do not require accessory high-energy molecules such as ATP for their signal, simplifying their use in a number of new reporter applications. Several features of *Gaussia* luciferase make it very attractive as a novel reporter system for studying gene expression.



Advantages of using *Gaussia* luciferase as a reporter gene:

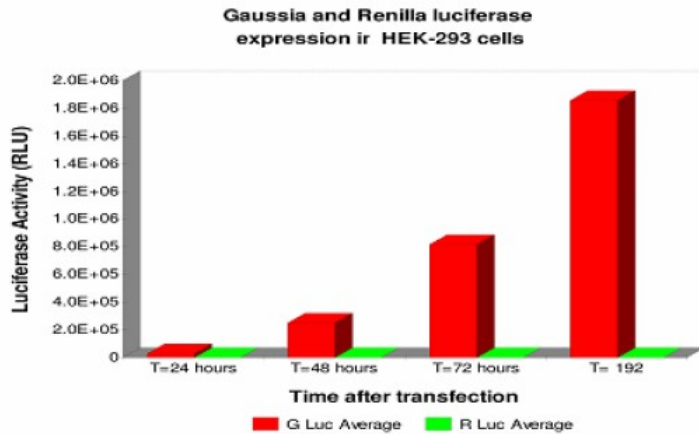
- *Gaussia* luciferase is up to 1000-fold brighter than native *Renilla reniformis* luciferase or firefly luciferase.
- *Gaussia* luciferase is stable at elevated temperatures.
- *Gaussia* luciferase is secreted into the media.

It is therefore necessary to only assay supernatants for enzyme activity without the need for lysing the cells. Considerable time is saved since time course experiments can be performed using the same group of transfected cells without lysing the cell

- Preliminary studies in mammalian cells indicate that luminescence in cells transfected with *Gaussia* luciferase expression vectors is greater than luminescence of cells transfected with *Renilla luciferase* expression vectors by at least three orders of magnitude.

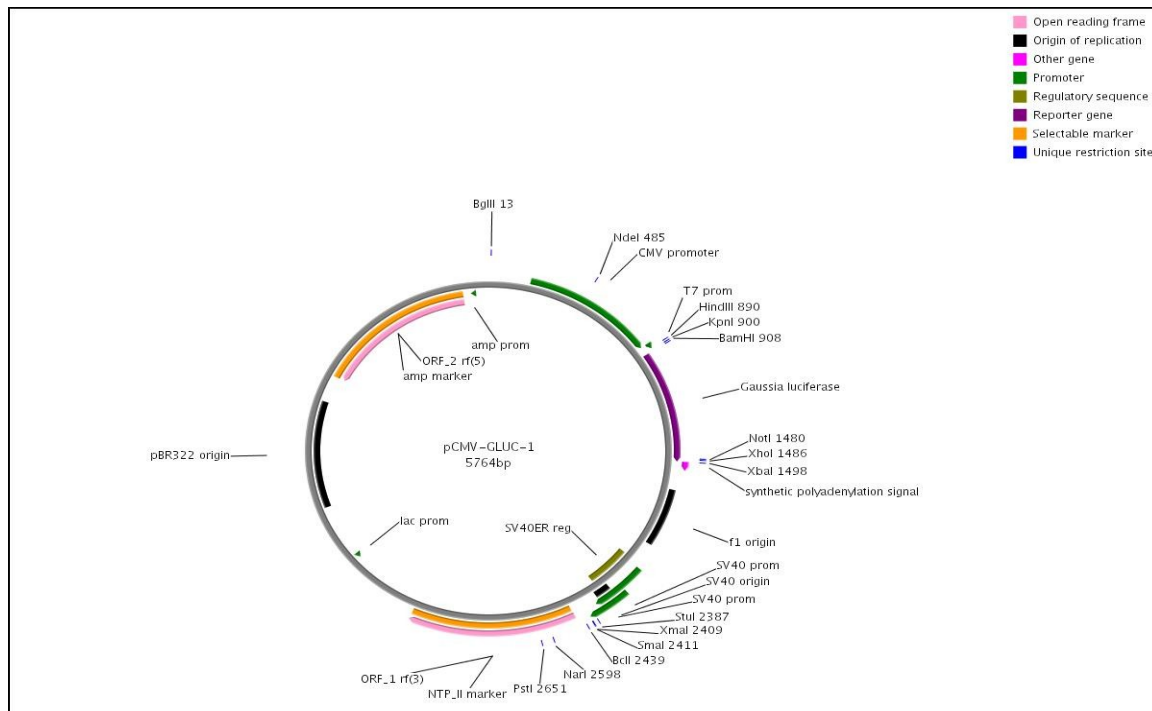
This makes it particularly useful for analyzing gene expression in hard to transfect cells or for studying regulation of weak promoters. The high luciferase activity is also an advantage for high throughput screening applications.

- *Gaussia* luciferase is extremely sensitive and able to detect 10⁻²¹ mol levels of enzyme
- The *Gaussia* luciferase assay kit stabilizes the flash signal emitted by the *Gaussia* luciferase thus making it possible to use it as a reporter gene for high throughput applications



A time course experiment to assess stability of the secreted *Gaussia* luciferase

HEK-293 cells were transfected with expression vectors expressing either secreted *Gaussia* luciferase or secreted *Renilla* luciferases under control of the CMV promoter. Cell supernatants were assayed for luciferase activity at different times after transfection up to 8 days. The results of this experiment suggest that the secreted *Gaussia* luciferase is thermostable and accumulates in the medium. *Renilla* luciferase activity in the transfected cells starts declining after 72 hrs. Thus use of *Gaussia* luciferase as a reporter gene permits experiments evaluating gene expression over a longer time period.



Features of the expression vector:

- pCMV-GLUC-1 (5764 bp)
- CMV promoter bases: 209-863
- Gaussia* luciferase gene: 907-1497
- T7 promoter bases: 864-882
- Polylinker bases: 889-907
- SP6 promoter: 1513-1530
- Synthetic polyadenylation site: 1497-1541
- SV40 promoter bases: 2082-2417
- SV40 origin of replication: bases 2196-2281
- Neomycin ORF : bases 2453-3247
- SV40 PolyA: bases 3302-3674
- ColE1 origin: bases 3934-4607
- Ampicillin ORF: bases 4752-5612
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Directions for use

The humanized *Gaussia* luciferase is secreted into the culture media and only the media need be assayed by the addition of native coelenterazine (do not use benzyl coelenterazine).

Gaussia luciferase Assay Protocol

The *Gaussia* Assay Reagent (GAR) is prepared freshly by diluting the coelenterazine stock (1:100 dilution) with the assay buffer. The assay is performed as following:

- 1- Add 50µl of GAR to 20 µl *Gaussia* luciferase sample (in Serum free DMEM or DMEM with 2% Serum) from microtiter or culture well samples
- 2- Mix well and read in luminometer.

The light with GAR is emitted stably for up to 45 minutes.

If coelenterazine without stabilizers is used, it is best in an injecting luminometer set to count from time=0 since the *Gaussia* luciferase is very high in turnover. After 10 seconds, the coelenterazine is consumed.

Related products

- UptiFectin-On DNA Transfection Reagent, [CK5060](#)
- Coelenterazine, native, [972333](#)

References

- [Xiaoli Dong, Paul Stothard, Ian J. Forsythe, and David S. Wishart "PlasMapper: a web server for drawing and auto-annotating plasmid maps" Nucleic Acids Res. 2004 Jul 1;32\(Web Server issue\):W660-4.](#)

The CMV promoter is covered by U.S. Patents 5,168,062 and 5,385,839 and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242

Gaussia luciferase is covered by US Patent # 6,232, 107 and IPO patents issued to Prolume Inc. Pittsburgh, PA. This plasmid is being sold for research purposes only.

Ordering information

Catalog size quantities and prices may be found at <http://www.fluoprobes.com>
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06

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