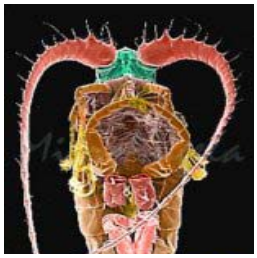
Gaussia princeps Luciferase



Intro

Gaussia princeps is the largest copepod that lives in the Hawaiian waters (Image by Microangela). *Gaussia* luciferase uses coelenterazine and its derivatives (Cat# 20001 to 20003) to catalyse the oxidative decarboxylation of coelenterazine to produce coelenteramide and light.

***Gaussia* luciferase products available:**

Vectors:

1. **pGLuc** (Cat# 20101) – pUC19-based plasmid with *Gaussia* luciferase sequence optimised for expression in bacterial cells
2. **pCDNA** (Cat# 20106) – pCDNA3-based plasmid with humanised *Gaussia* luciferase sequence; codon optimised for expression in mammalian cells

Recombinant:

1. ***Gaussia* luciferase 45% purity** (Cat# 20107 and 20108) – crude preparation, low grade material ideal as a teaching aid. Suggested experiments and protocols are available - please contact us at info@luxbiotech.com

Biotinylated:

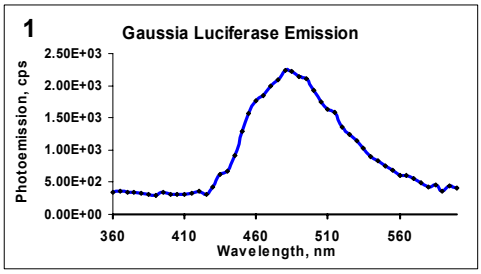
2. **Biotinylated luciferase – 2x brighter than normal *Gaussia* luciferase** for sensitive detection of proteins, DNA, etc. - available alone (Cat# 20109) or as a kit including buffers and coelenterazine (Cat# 20110) (see separate product sheet)

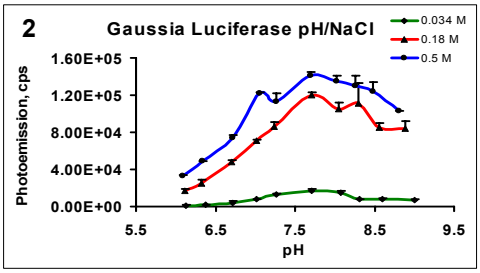
pCMV-based *Gaussia* luciferase products are also available but require approximately three weeks to despatch

Technical information:

Gaussia princeps luciferase is the **smallest luciferase** isolated to date.
It has a **spectral peak** at **480 nm** (figure 1)
It is **sodium dependent** (figure 2).
Similarly to *Renilla* luciferase it is **ATP independent**.
The **specific activity** of this luciferase in the presence of high concentrations of coelenterazine (10µM) is extremely high: **1.24 x 10^{16} Qps/mg** (Quanta per second per milligram).
Gaussia luciferase contains a **signal peptide**, and the protein is therefore secreted from mammalian cells. The signal peptide does not function in *E. coli.*
Note that benzyl coelenterazine (h-CTZ) is not recommended for use with *Gaussia* luciferases – use native coelenterazine instead (n-CTZ).

Analysis	
Length	185 aa
Molecular Weight	19899
Molar Extinction coefficient	8290
Isoelectric Point	6.88
Charge at pH 7	-0.15
Amino Acid(s) % frequency	
Charged (RKHYCDE)	33.51
Acidic (DE)	12.97
Basic (KR)	12.97
Polar (NCQSTY)	21.08
Hydrophobic (AILFWV)	35.68
C Cys	5.95 (11)





The features of this luciferase include:

pH resistance (surviving a pH range of 3-11);
Good thermostability (up to 60°C and approx. 20% recovery following a 15 minute incubation at 99°C);
Activity even in the presence of non-ionic detergents (1-5% nonionic detergents (NP-40, Triton X-100, Triton X-114, CHAPSO);
Resistance to cholate, deoxycholate etc. and ability to recover activity after treatment with 7M guanidine chloride or 8M urea+NP-40;
Greater brightness compared to other luciferases. Following transformation of Chinese hamster ovary cells, native ***Gaussia** princeps* luciferase gave a **15-fold increased** luminescence compared to commercially available *Renilla* luciferase and **750-fold greater** luminescence after human codon optimization **(see data below)**;
Peak light output: pH=7.8 in 500 mM NaCl

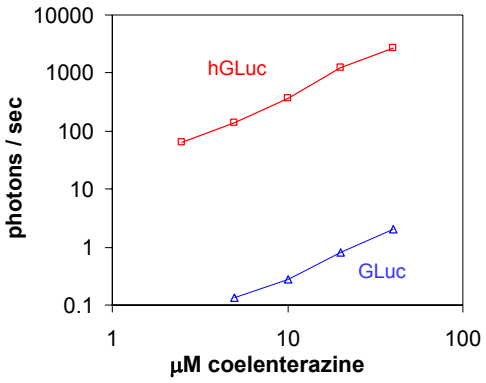
Comparison of Gaussia luciferase to other commonly used luciferases

SPECIES	LUCIFREASE	SIZE	QUANTUM YIELD	WAVELENGTH	ATP DEPENDENCY	SUBSTRATE
Photynus pyralis (Firefly)	FLuc	550 aa	>88%	562 nm	YES	D-luciferin
Renilla reniformis (Sea pansy)	RLuc	311 aa	>6%	480 nm	NO	coelenterazine
Pleuromamma xiphias (Copepod)	PLuc	198 aa	Not known	480 nm	NO	coelenterazine
***Gaussia princeps* (Copepod)**	**GLuc**	**185 aa**	1.6x10^{16}Qps/mg	**480 nm**	**NO**	**coelenterazine**

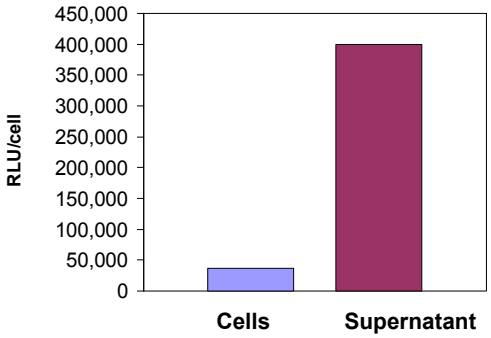
DATA

Expression of Gaussia princeps luciferase (GLuc) *in vitro* and *in vivo*

Humanised GLuc shows 2000 fold higher bioluminescence than wild-type GLuc when expressed in mammalian cells. Tannous *et*

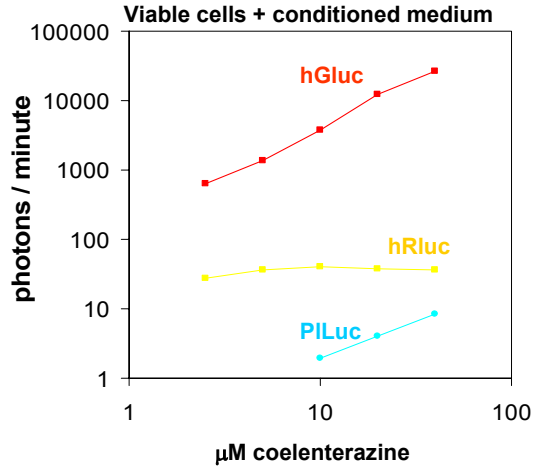


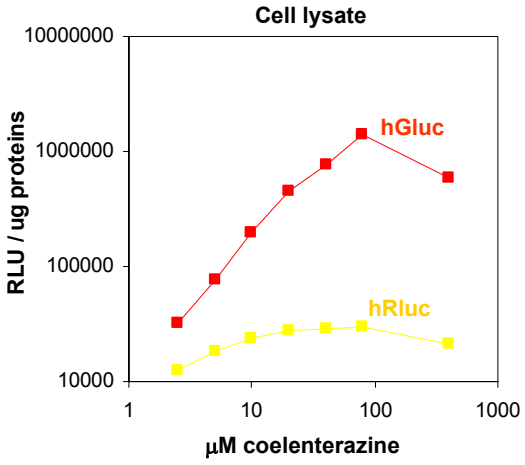
Gaussia luciferase is naturally secreted from expressing cells in active form.



Effect on luminescent signal with increasing coelenterazine concentration

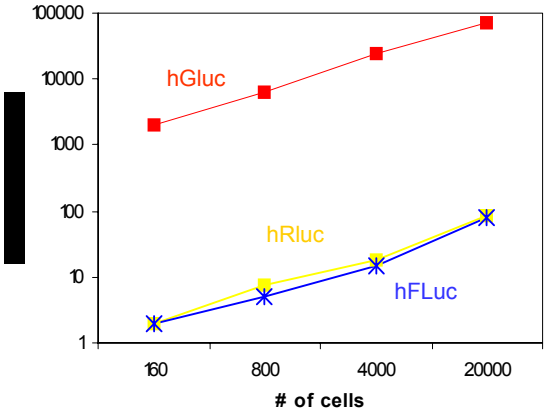
hGLuc shows higher bioluminescent signal than hRLuc or PlLuc at any giving coelenterazine dose and despite its secretion, the intracellular signal is still 50-fold higher than hRluc in mammalian cells. Tannous *et al* 2005.



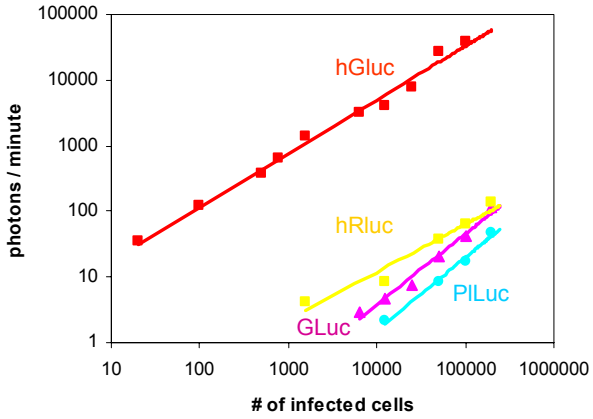


Activity of different luciferases compared to GLuc *in vitro*

Humanized *Gaussia* luciferase is over 1000-fold more sensitive than humanized *Renilla* or humanized firefly luciferases, and the wild-type *Gaussia* luciferase shows similar sensitivity as the wild-type *Pleuromamma* luciferase when all expressed in mammalian cells under similar conditions. Tannous *et al* 2005.



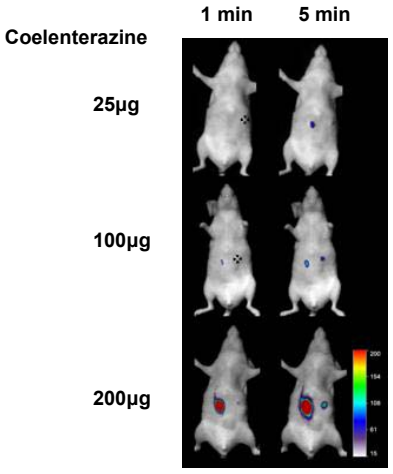
Lipofectamine transfection

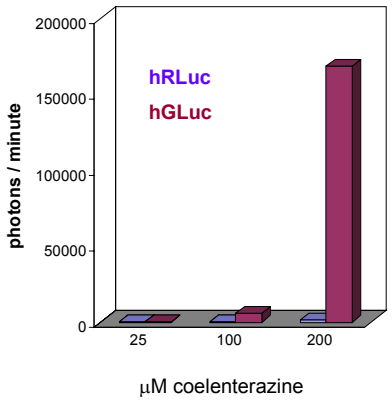


HSV-1 amplicon vector transfection

Activity of GLuc compared to RLuc *in vivo*

Humanized *Gaussia* luciferase shows 200-fold higher bioluminescence than humanized *Renilla* luciferase *in vivo* using a nude mouse model of subcutaneous glioma tumors. Tannous *et al* 2005.





Note on usage of Nanolight genes and note on usage

These vectors are sold under license only for non-commercial research purposes. This means:

1. **Purchaser does not have the right to transfer the gene.**
2. **Nanolight retains all rights to any coding sequence modifications of mutations upon the coding sequence.**
3. **Plasmid is supplied for non-commercial research purposes only, specifically excluding high throughput drug screening or uses that would result in commercial applications.**
4. **Commercial licenses are available at reasonable costs and conditions.**

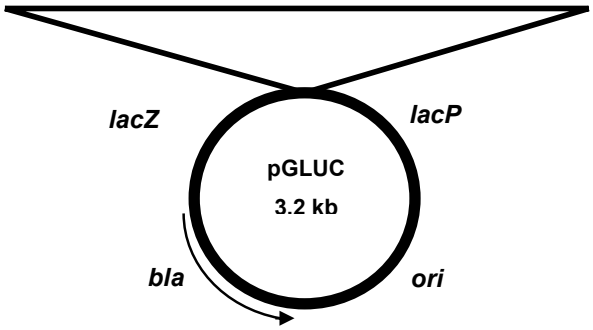
Plasmid map

The schematic of the native *Gaussia* luciferase in pUC19 vector (Cat# 20101) is shown below. The humanised vector information is in a separate document.

pGLUC (Cat# 20101)

Native *Gaussia* luciferase PCR product

GAA TTC AAA ATG AAA CCA ACT GAA AAC AAT GAA GAT TTC AAC ATT GTA GCT GTA
GCT AGC AAC TTT GCT ACA ACG GAT CTC GAT GCT GAC CGT GGT AAA TTG CCC GGA
AAA AAA TTA CCA CTT GAG GTA CTC AAA GAA ATG GAA GCC AAT GCT AGG AAA GCT
GGC TGC ACT AGG GGA TGT CTG ATA TGC CTG TCA CAC ATC AAG TGT ACA CCC AAA
ATG AAG AAG TTT ATC CCA GGA AGA TGC CAC ACC TAT GAA GGA GAC AAA GAA AGT
GCA CAG GGA GGA ATA GGA GAG GCT ATT GTT GAC ATT CCT GAA ATT CCT GGG TTT
AAG GAT TTG GAA CCC ATG GAA CAA TTC ATT GCA CAA GTT GAC CTA TGT GTA GAC
TGC ACA ACT GGA TGC CTC AAA GGT CTT GCC AAT GTG CAA TGT TCT GAT TTA CTC
AAG AAA TGG CTG CCA CAA AGA TGT GCA ACT TTT GCT AGC AAA ATT CAA GGC CAA
GTG GAC AAA ATA AAG GGT GCC GGT GGT GAT CAT CAC CAT CAC CAT CAC TTA ***TCT***
AGA



The primers were designed to introduce an *Eco*RI site at the 5' end and a *Xba*I site at the 3' end of the fragment (shown in *italic* on the sequence). The 541 bp *Eco*RI/ *Xba*I digested PCR product was ligated into similarly linearized pUC19. The underlined ATG is the first codon of the LUC coding sequence. Six histidine codons were added on the 3' end of the gene to facilitate purification of protein products.

References:

Serganova I, Moroz E, Moroz M, Pillarsetty N and Blasberg R. (2006) Non-invasive molecular imaging and reporter genes. *Central European Journal of Biology*. **1**: pp. 88-123.

Tannous BA, Kim DE, Fernandez JL, Weissleder R and Breakefield XO. (2005) Codon-optimized Gaussia luciferase cDNA for mammalian gene expression in culture and *in vivo*. *Mol. Ther.* **11**: pp. 435–443.

Verhaegen M and Christopoulos TK. (2002) Recombinant *Gaussia* luciferase. Overexpression, purification and analytical application of a bioluminescent reporter for DNA hybridization. *Anal. Chem.* **74**: pp. 4378–4385.