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Gaussia Princeps Luciferase -

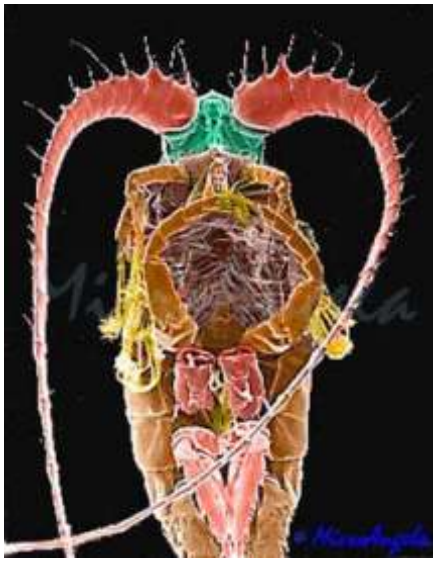
A Novel luciferase 1000-fold brighter than firefly and Renilla luciferase

Products :

1) pGluc vectors - expressing native secreted Gaussia luciferase
2) pGluc-Kdel vectors - expressing intracellular Gaussia luciferase
3) Gaussia Luciferase assay reagents - (Including reagents for high throughput applications)

Related Products :

1) psiScreen vector Efficient and rapid screening of siRNAs for effectiveness in gene silencing
2) Transfection reagents for efficient co-delivery of plasmid DNA and siRNA
3) Renilla Luciferase Assay Reagents
4) Firefly Luciferase Assay Reagents



New Products :

Gaussia Luciferase Assay System -

Gaussia luciferase is a novel secreted luciferase that is approximately 1000 times brighter than Renilla and firefly luciferases

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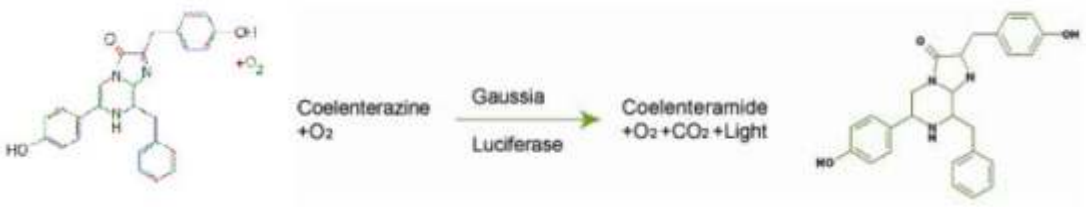
Product Profile :

Gaussia luciferase, a novel reporter for gene expression, is the smallest and brightest known luciferase **(approx. 1000-fold brighter than firefly or Renilla luciferases!) Great for studying weak promoters or hard-to-transfect cells.**

Advantages :

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 sold by Targeting Systems stabilizes the flash signal emitted by the Gaussia luciferase thus making it possible to use it as a reporter gene for high throughput applications

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Gaussia luciferase-based products offered by Targeting System:

Expression vectors for studying regulation of gene expression
Four vectors encoding Gaussia luciferase are presently being offered to study reporter gene expression :

1) Gaussia expression vectors secreting Gaussia luciferase :

• **pCMV-Gluc-1** Humanized Gaussia luciferase with secretion signal. This positive control vector is very useful in evaluating the efficiency of transgene expression using Gaussia luciferase as a reporter. This vector has both Ampicillin resistance and Neomycin resistance. Therefore it can be easily propagated in E. coli and can be used to establish stable cell lines expressing Gaussia luciferase.

• **pGluc-Basic-1 vector** A promoterless vector with a MCS site upstream of the humanized Gaussia luciferase coding sequence (with secretion signal). This vector is designed for promoter analysis and will express secreted Gaussia luciferase

2) Gaussia expression vectors expressing intracellular Gaussia luciferase :

• **pCMV-Gluc-kdel -** Humanized Gaussia luciferase with C-terminal KDEL endoplasmic reticulum retention signal for intracellular expression. The KDEL sequence is an endoplasmic reticulum retention signal which causes retention of the Gaussia luciferase in the endoplasmic reticulum and results in high levels of intracellular luciferase expression.

• **pGluc-Basic-kdel vector -** A promoterless vector with a MCS site upstream of the native Gaussia luciferase coding sequence. This vector is designed for promoter analysis and will express high levels of intracellular Gaussia luciferase.

All plasmid vectors are provided at a concentration of 1 µg/µl

3) Gaussia luciferase-based system for screening siRNAs for their effectiveness in gene silencing :

The siScreen vector enables easy and fast screening of siRNA libraries for their effectiveness in silencing the target gene of interest. When using this system the target gene of interest is subcloned into the multiple cloning sites downstream of the Gaussia luciferase stop codon. Co-transfection of this vector with an effective siRNA against the target gene results in a decrease in luciferase activity. The decrease in luciferase activity is directly proportional to the effectiveness of the siRNA. Since the target gene is cloned after the gaussia luciferase stop codon, expression of gaussia luciferase is unaffected since the target protein is never expressed. However, an effective siRNA against the target gene will result in degradation of the gaussia luciferase-target gene mRNA resulting in a decline in luciferase activity. Highly efficient co-transfection of siRNA with plasmid DNA can be achieved using the targefect-F2 reagent. Assay of gaussia luciferase in media supernatants is performed using the GAR-1 reagent. The effectiveness of this system for screening siRNAs has been well demonstrated using the tumor suppressor p53 gene as an example. For more details refer to the brochure on the siScreen system on our website www.targetingsystems.com. A major advantage of the siScreen systems is that it enables rapid screening of siRNAs using a simple one-step luciferase assay and eliminates the need for performing cumbersome Western blots or real time PCR measurements to assess gene silencing.

4) Assay reagents for Gaussia luciferase :

Three types of luciferase assay reagent are currently offered for measurement of Gaussia luciferase in various applications -

• **GAR1 :** This reagent is used for measurement of Gaussia luciferase in applications when the luciferase activity is measured immediately after addition of the assay reagent to the luciferase containing supernatant. This reagent can be used for assaying gaussia luciferase or renilla luciferase activity in cell-supernatants or in cell lysates. This reagent is compatible

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for measuring luciferase activity in cells lysed with either the lysis buffer from Targeting Systems or the Promega RLAR lysis buffer or passive lysis buffers.

• **GAR-2 :** Gaussia luciferase assay reagents for high throughput applications requiring a stable bioluminescent signal in the first 15-30 mins of the assay. The bioluminescent signal using this assay reagent is stable for the first 15 mins and then declines slowly. The half life of the bioluminescent signal is 30 mins. The intensity of the bioluminescent signal is less than that obtained with the GAR-1 reagent because of the effect of the stabilizers in the GAR-2 reagent.

• **GAR-B2 :** Luciferase assay reagent with increased intensity of the bioluminescent signal.

The intensity of the bioluminescent signal using this reagent is approximately 10-times more that that obtained using the GAR-1 reagent due to the addition of components that increase the brightness of the luciferase signal. This reagent is highly recommended for applications wherein greater sensitivity is desired e.g analysis of weak promoters or studies involving hard-to transfect cells. This reagent is not recommended for high throughput applications as the bioluminescent signal using this reagent is not very stable.

Renilla Luciferase Assay

Targeting Systems offers expression vectors and assay reagents for a novel improved, green-emitting, secreted Renilla luciferase mutant which is far brighter than native Renilla luciferase and exhibits better stability both in vitro and in vivo- A tremendous advantage both for HTS and for in vivo imaging applications. For more information visit our website www.targetingsystems.net

References citing Gaussia luciferase:

1. Jinming Yang, Snjezana Zaja-Milatovic, Yee-Mon Thu, Francis Lee, Richard Smykla, and Ann Richmond (2009) Molecular determinants of melanoma malignancy: selecting targets for improved efficacy of chemotherapy. Mol. Cancer Ther., Mar 2009; 8: 636 - 647.

2. Tannous BA (2009) Gaussia luciferase reporter assay for monitoring biological processes in culture and in vivo. Nature Protocols 4, - 582 - 591 (2009)

3. Wurdinger T, Badr C and Tannous B (2008) Gaussia luciferase blood level as an index of cell growth and proliferation

4. Wurdinger T, Badr C, Pike L, de Kline R, Weissleder R, Breakerfield X, and Tannous B (2008) A secreted luciferase for ex vivo monitoring of in vivo processes. Nature Methods - 5, 171 - 173

5. Cheng G and Davis RE (2007). An improved and secreted luciferase reporter for schistosomes. Molecular & Biochemical Parasitology 155 (2007) 167–171.

6. Drug Discovery Today: Technologies Volume 1, Issue 4, December 2004, Pages 357-364

7. Nicola Ternette,1 Daniela Stefanou,1 Seraphin Kuate,1 Klaus Überla,1 and Thomas Grunwald (2007) Expression of RNA virus proteins by RNA polymerase II dependent expression plasmids is hindered at multiple steps. Virol J. 2007; 4: 51.

8. Guofeng Chenga,1, Leah Cohenb,1, Claudette Mikhli b, Marzena Jankowska-Anyszka c (2007) In vivo translation and stability of trans-spliced mRNAs in nematode embryos.

Relevant interesting papers on new applications of Gaussia luciferase

1. Development of a Dual-Luciferase Reporter System for In Vivo Visualization of MicroRNA Biogenesis and Posttranscriptional RegulationJi Young Lee, Soonhag Kim, Do Won Hwang, Jae Min Jeong, June-Key Chung, Myung Chul Lee, and Dong Soo Lee. J. Nucl. Med., Feb 2008; 49: 285 - 294.

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Comparision of Gaussia Luciferase with firefly and Renilla Luciferase in HEK-293 cells :

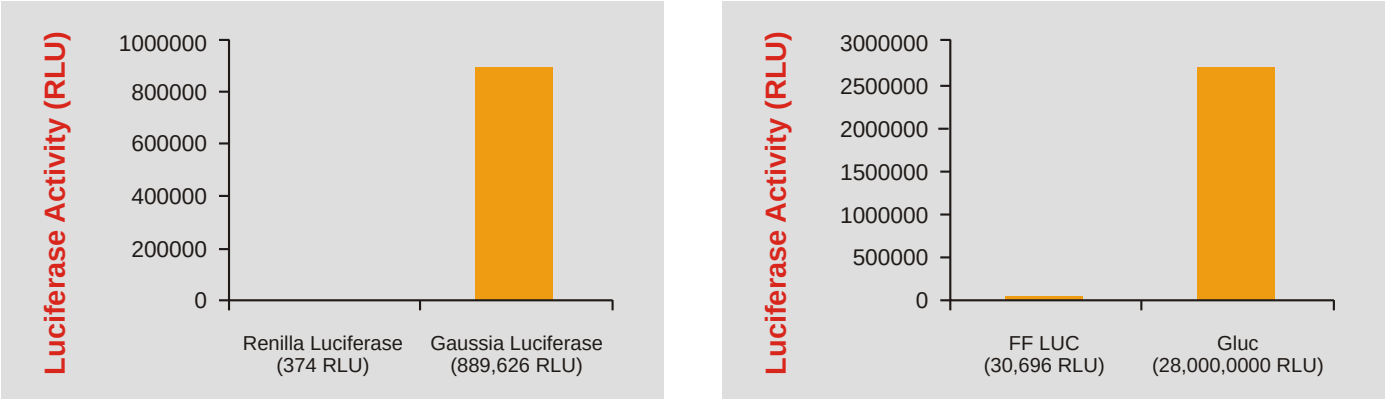
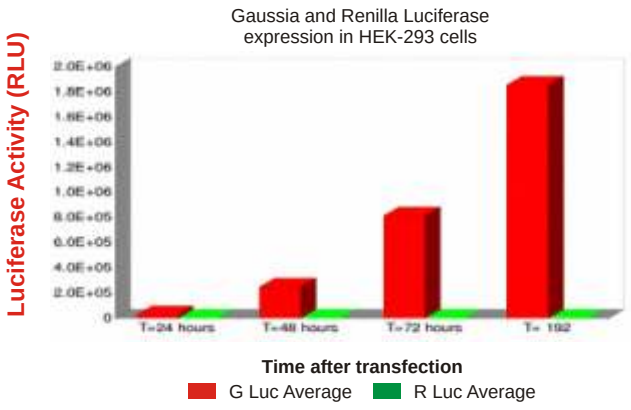


Figure 1 : Expression of Gaussia Luciferase, Firefly luciferase and Renilla luciferase in HEK-293 cells transfected with CMV expression vectors expressing either Gaussia or Renilla luciferases

The transfected cells express the Gaussia and Renilla luciferases as secreted products and firefly luciferase intracellularly. Gaussia luciferase has a 14 amino acid secretory signal which enable sufficient secretion of the expressed enzyme in mammalian cells. The Renilla expression vector (Novagen, pMLuc vector) had an interleukin 2 secretory signal cloned upstream of the Renilla luciferase coding sequence. In this experiment HEK-293 cells set up in 6-well dishes were transfected with equivalent amounts of Gaussia or renilla or firefly luciferase expression vectors (with the CMV promoter) using the Targefect F-1 reagent (Targeting Systems). Aliquots of the supernatant media were collected and assayed for luciferase expression 48 hrs after transfection. To determine the firefly luciferase activity cells were lysed at 48 hrs post transfection. The data shown (average of quadruplicate transfections) indicate luciferase activity in 20 µl of supernatant media from transfected HEK-293 cells (total volume of supernatant media per dish was 3ml. The data shown above demonstrates that luciferase activity in media supernatants of HEK-293 cells transfected with Gaussia luciferase (889626 RLU) was 2500 times more than luciferase activity in supernatants of cells transfected with Renilla luciferase (374 RLU). In a similar experiment (Fig 1A, top panel) total luciferase activity per well in cells transfected with gaussia luciferase expression vectors (28 million RLU) was 912 times higher than luciferase activity in cells transfected with firefly luciferase expression vectors (30,696 RLU).

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 thermo stable 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.

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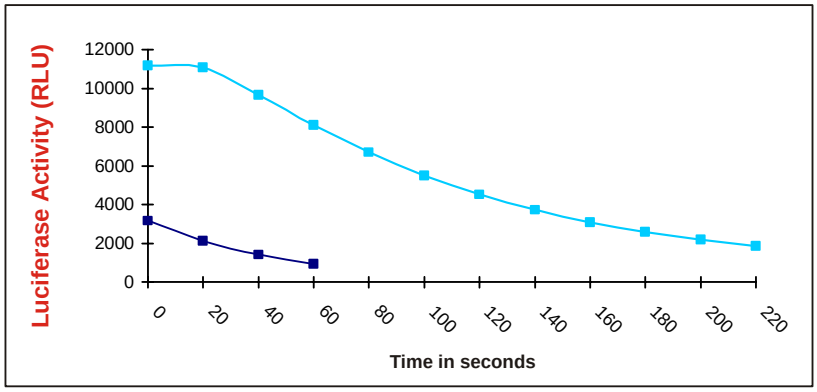
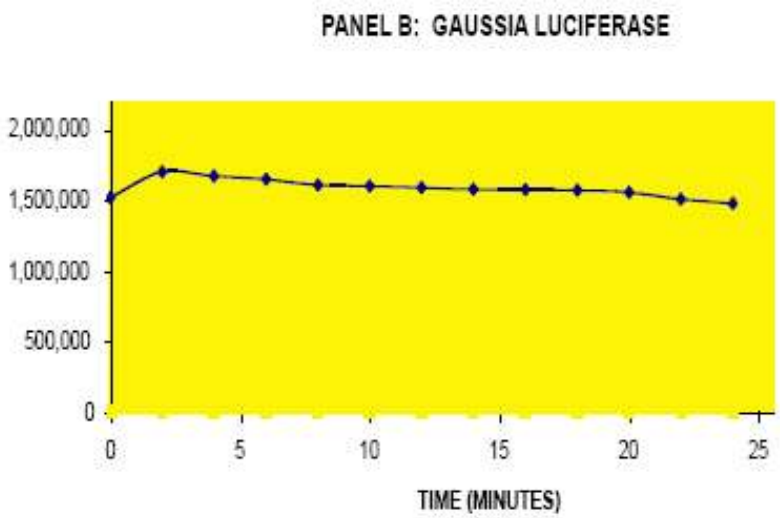
Reagents for assay of Gaussia luciferase :

Although the Gaussia luminescent signal is very bright, it decays rapidly. However, we have observed that inclusion of certain stabilizing factors in the composition of the assay reagent can result in stabilization of the luminescent signal, making it suitable for use as a reporter gene. Assay of Gaussia luciferase activity using Promega's Renilla assay reagent (see graph below) results in a 60% drop in activity in 1 min, use of the Mightylight renilla assay reagent from Novagen resulted in a 60% drop in 1 minute (data not plotted) and use of the Targeting Systems assay reagent GAR-1 results in a less than 10% drop in luciferase activity over a 1 minute time interval. Data shown represents the average of triplicate determinations.



Comparison of GAR-1 (Targeting Systems) and Promega's Renilla assay system (part of Dual luciferase) for assay of Gaussia luciferase (Note: the initial reading (t=0) was taken 20 seconds after addition of GAR reagent). 20 µl of Gaussia luciferase containing supernatants were mixed with either 50 µl of Gaussia luciferase assay reagent (dark blue squares) or 100 µl Promega's Renilla luciferase assay reagent. The Gaussia luciferase assay reagent (GAR-1) offered by Targeting Systems gives a brighter and more stable luminescent signal compared to Promega's Renilla luciferase assay reagent

GAR-2 - A more stable version of the Gaussia luciferase assay reagent for high throughput screening applications :



Stability of the GLuc bioluminescent signal using the GAR-2 reagent:
Using the GAR-2 version of the Gaussia luciferase assay reagent the bioluminescent signal remainsvery stable (Panel A). This reagent is useful for HTS applications in which a large number of samples need to be assayed.

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Gaussia Luciferase Assay Protocols :

GAR-1/GAR-B2 Assay Protocol

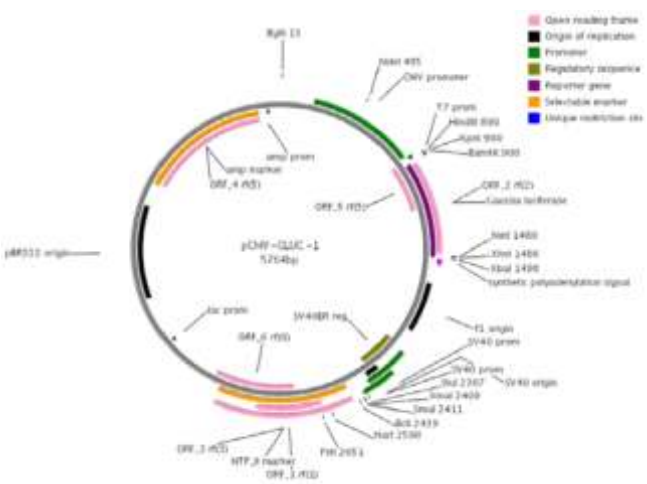
To 20 µl of Gaussia luciferase samples, add 50 µl of the GAR-1 reagent. Mix well and read in the luminometer.

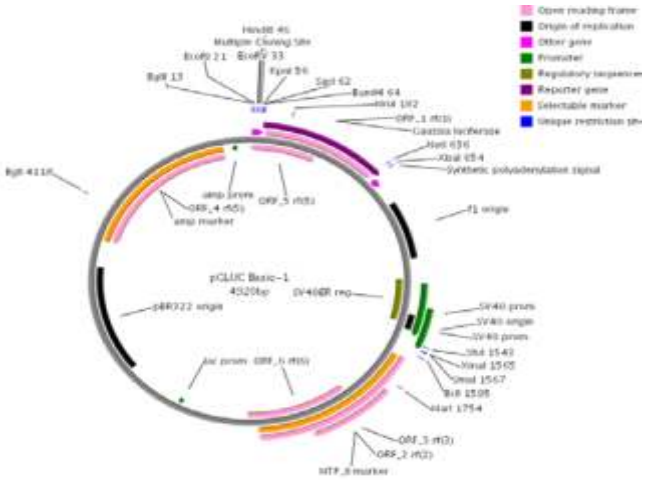
GAR-2 Assay Protocol

To 20 µl of the Gaussia luciferase samples, add 8 µl of the GAR-stabilizer, mix and add 50 µl of the GAR-2 reagent. Mix well and read in luminometer.

Gaussia Luciferase Expression Vectors :

Plasmid Map of pCMV-GLUC-1





Features of 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

Features of pGLUC Basic-1 (4920 bp)

- T7 promoter bases : 20-38
- Polylinker bases : 12-63
- Gaussia luciferase reporter gene : 63-653
- SP6 promoter : 669-686
- Synthetic polyadenylation site : 653-697
- SV40 promoter bases : 1253-1573
- SV40 origin of replication : bases 1352-1437
- Neomycin ORF : bases 1609-2403
- SV40 PolyA : bases 2458-2830
- ColE1 origin : bases 3090-3763,
- Ampicillin ORF : bases 3908-4768

The plasmid map shown above was constructed with the help of the following link :
http://wishart.biology.ualberta.ca/PlasMapper/jsp/displayPlasmidMap.jsp?fileName=plasMap115_1101977745436.jpg&fileFormat=jpg

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.

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Gaussia Luciferase is covered by US patent #6,232,107 and IPO patents issued to Prolume Inc. This Plasmid is being sold for research purposes only. Commercial users/ For profit companies and foundations will require a license from Prolume (www.nanolight.com) for the use of Gaussia gene after 180 days of evaluation and from the University of Iowa Research Foundation for the use of the CMV promoter.

Product Pricing

Gaussia Luciferase-based products

Plasmid Expression vectors, Lenti-Pleamids, LentiGlo –ready-to –use lentivirus expressing Gaussia luciferase

Catalog No.	Size	Description	Price
		Gaussia Luciferase Expression Vectors:	
GL-001	25 ug	pCMV-GLuc : Expresses native Gaussia luciferase under CMV promoter	$ 299.00
GL-002	25 ug	pGLuc-Basic : Promoterless vector expressing Gaussia luciferase	$ 299.00
GL-K001	25 ug	pCMV-GLuc-KDEL: Expresses intracellular Gaussia luciferease under control of CMV promtoter due to presence of KDEL (endoplasmic reticulum retention sequence)	$ 299.00
GL-K002	25 ug	pBasic-GLuc-KDEL :Promoterless expression vector expressing intracellular Gaussia luciferase	$ 299.00
GL-GFP	25 ug	plenti-GLuc-EGFP :Lenti vector expressing Gaussia luciferase under control of CMV promoter, co-expressing EGFP using an IRES.	$ 600.00
		SINGLE ASSAYS	
GAR-1	1000 assays	Gaussia Luciferase Assay	$ 375
GAR-2 0090A	1000 assays	Gaussia Luciferase Assay (Stable signal)	$ 400
GAR-2B	1000 assays	Gaussia lLuciferase Assay (Brighter, Stable version)	$ 420
		DUAL LUCIFERASE ASSAYS	
DLAR-1 DLAR-4	1000 assays 1000 assays	Gaussia-Red Firefly Luciferase Cypridina-Gaussia Luciferase	$ 850 $ 900
		TRIPLE LUCIFERASE ASSAYS	
TLAR-2	1000 assays	Cypridina Luciferase Gaussia Luciferase, red Firefly Luciferase Assay reagent	$ 1000

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Lentiviral Vectors Expressing Gaussia Luciferase

Catalog No.	Product Name	Description	Price
LP-07	PLenti-CMV-GLuc-IRES-GFP	Lentivirus expressing Gaussia luciferase and GFP, pLenti-CMV-Gluc-IRES-EGFP plasmid, 1000 assays of the Gaussia luciferase assay reagent GAR-1	$2000
Lenti-CUstom	Lenti-Custom	Any of the above expressing luciferase under your promoter of interest (subject to availability)	$2500
LP-01	pLenti-Basic-Gluc	Promoterless Lenti plasmid expressing Gaussia luciferase, packaging mix, 1000 assays of luciferase assay reagent Cypridina-Gaussia Luciferase	$1650

References citing luciferase reporter products from Targeting Systems

1.Jinming Yang, Snjezana Zaja-Milatovic, Yee-Mon Thu, Francis Lee, Richard Smykla, and Ann Richmond (2009) Molecular determinants of melanoma malignancy: selecting targets for improved efficacy of chemotherapy. Mol. Cancer Ther., Mar 2009; 8: 636 - 647.

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