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Gaussia Luciferase Assay reagent (GAR-1)

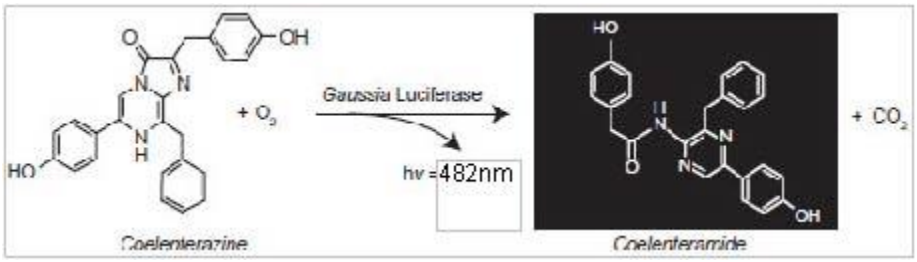
Catalog no.	Size	Description	Price
GAR-1	1000 assays	Gaussia luciferase assay reagent	$375.00

Please call for special pricing on bulk reagent purchase.

Description:
br /> The GAR-1 Assay Kit is used for measurement of Gaussia luciferase activity in a standard (non-highthroughput format wherein samples can be measured quickly in a microplate reader or regular tube luminometer but long-term stability of the bioluminescent signal is not required. For applications such as high throughput screening we have developed assay reagents (GAR-2 and GAR-2B) which provide excellent stability of the bioluminescent signal due to incorporation of stabilizers. The GAR-2B reagent also provides a brighter signal intensity of the luciferase signal.

About Gaussia Luciferase:
Gaussia Luciferase (GLuc) is a luciferase from the marine copepod Gaussia princeps (1,2). This luciferase, which does not require ATP, catalyzes the oxidation of the substrate coelenterazine in a reaction that produces light, and has considerable advantages over other luminescent reporter genes such as secretablity and a much brighter signal intensity in addition to excellent stability of the bioluminescent signal (approximately 10% decay in an hour. The luminescence measured from the supernatant of cultured cells transfected with a plasmid expressing GLuc is proportional to the amount of enzyme produced, which in turn, reflects the level of transcription. Alternatively a cell lysate can be used for the assay. Although most of the activity is secreted, the high sensitivity of Gaussia luciferase allows measurements from the cell lysates as well.

Figure 1: Photo-oxidation of coelenterazine catalyzed by Gaussia luciferase



Assay for secreted Gaussia luciferase actrivity:

Figure 1 : E xpression of G aussia Luciferase, Firefly luciferase and Renilla luciferase in H E K-293 cells transfected with CMV expression vectors expressing either G aussia or R enilla 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

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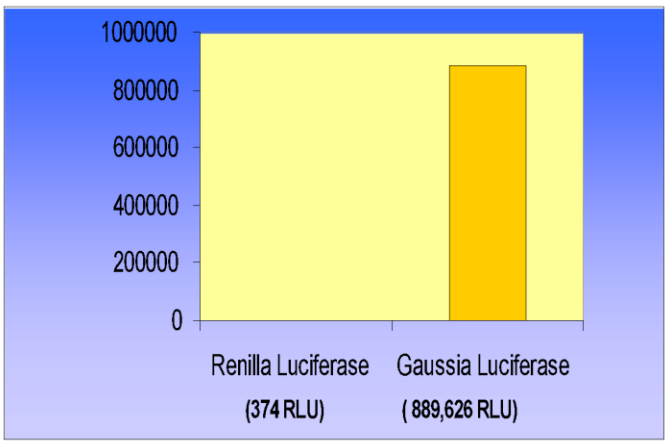
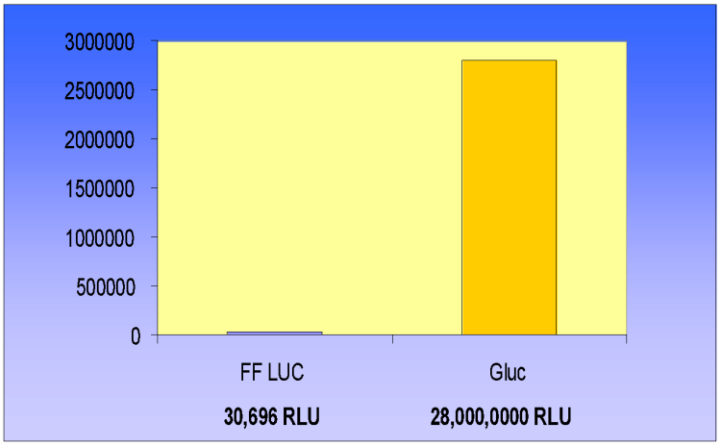
promoter)
using the Targefect F-1 reagent (Targeting Systems). Aliquots of the supernatant media were collected and assayed for luciferase expression 48 hrs after transfection. To determine the firefly luciferase activity cells were lysed at 48 hrs post transfection. The data shown (average of quadruplicate 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).





HEK-293 cells transfected with CMV-GLUC show ***2,378*** *times greater luminescence* compared to cells transfected with CMV-Rluc. (Data shown is average of quadruplicate determinations and represents luciferase activity in 20 ul of cell supernatant). Constructs used expressed either Renilla luciferase (secreted) or Gaussia luciferase under control of the CMV promoter.. Transfections were performed using the *Targefect F-1* reagent and cells supernatants assayed for luciferase activity 48 hrs post transfection

Luciferase activity in cells transfected with Gaussia luciferase is **912 times higher** than luciferase activity in cells transfected with firefly luciferase
HEK-293 cells grown in 6-well dishes and transfected with 2 ug of CMV-Fluc or CMV-GLuc and 4 ul of the *Targefect F-1* reagent. Data presented is the mean of triplicate determinations and represents only 10% of the total luciferase activity in each well.

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Figure 2A: Kinetics of of the mutant Gaussia luciferasc (pCMV-GLuc-Stable) bioluminescent signal using the GAR-1 reagent: Measurement of Gaussia luciferase activity in supernatants of cells (transfected with Gaussia luciferase expression vectors) using GAR-1 reagent from Targeting Systems. The Gaussia luciferase assay reagents (50 ul) was added to sample wells 5 ul of supernatant medium from HEK293 cells transfected with the Targeting Systems pCMV-*GLuc-stable* plasmid. Luciferase output was measured every 5 mins. Data shown is average of triplicate determinations. When assayed with the GAR-1 reagent the intensity of the bioluminescent signal of mutant Gluc is comparable to that of native Gluc but the bioluminescent sigfnal is very stable (half life greater than 1 hour making it well suited for HTS applications

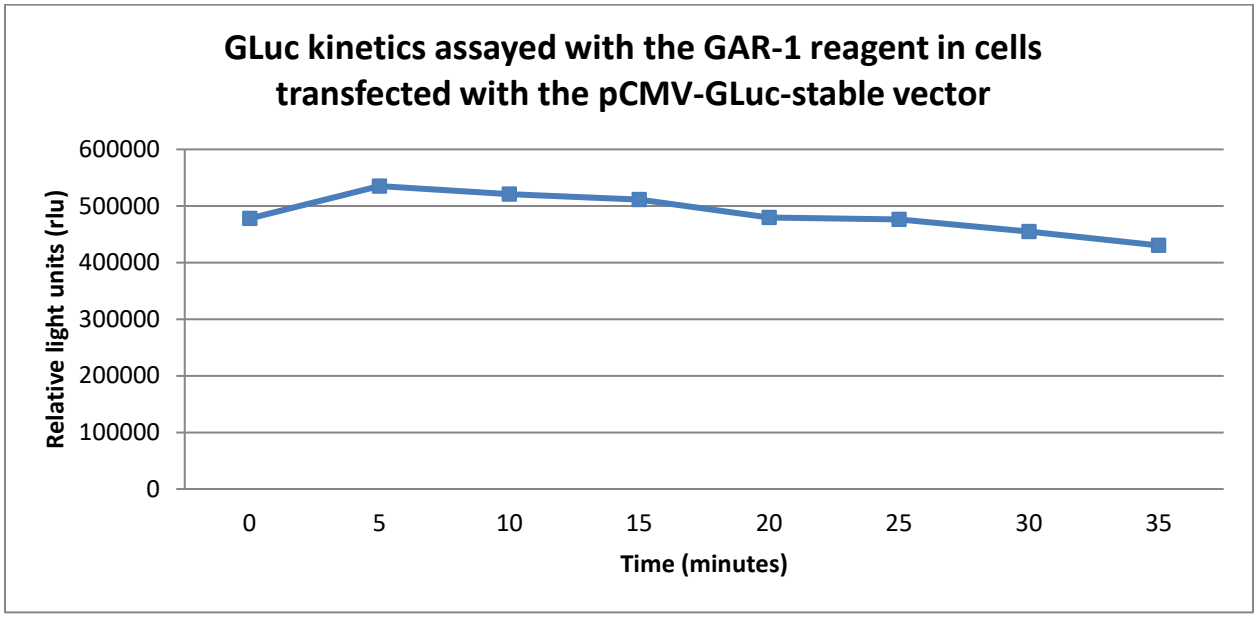

Figure 2B: Kinetics of of the Native GLuc bioluminescent signal using the GAR-1 reagent: Measurement of Gaussia luciferase activity in supernatants of cells (transfected with Gaussia luciferase expression vectors) using GAR-1 reagent from Targeting Systems (teal color line) and comparison with measurements obtained using the Renilla luciferase assay reagent form another commercial vendor (dark blue line).
Note: Data presented is average of triplicate determinations measured on a Turner TD2020 luminometer.

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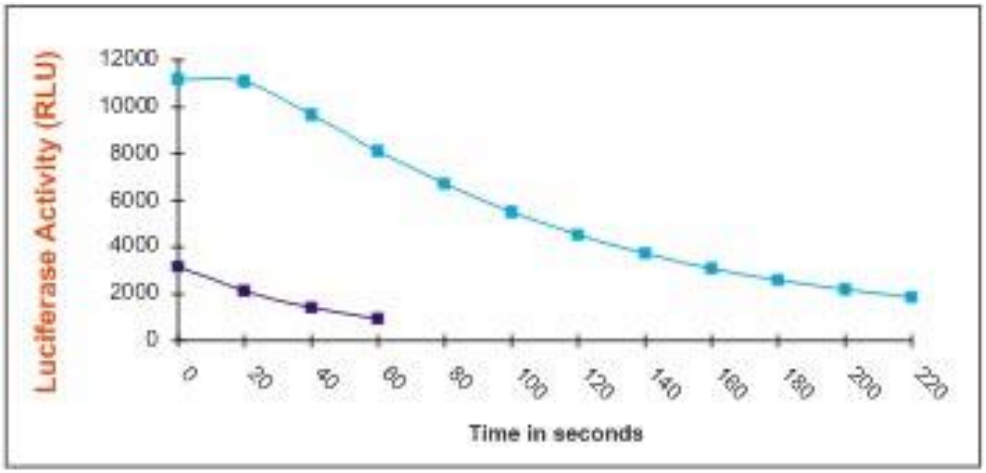

Advantages:

- Native Gaussia Luciferase (GLuc) possesses a natural secretory signal and upon expression is efficiently secreted into the cell medium. Cell-lysis not necessary for assaying the luciferase
- Gaussia Luciferase generates over 1000-fold higher bioluminescent signal intensity, than commonly used Firefly and Renilla Luciferases, making it an ideal transcriptional reporter (1).
- GLuc shows the highest reported activity of any characterized luciferases (1).
- The secreted protein is thermostable and has extremely high activity in light production allowing for very sensitive assays (1,2).
- The GLuc-containing samples (i.e. growth media or cell lysates after transfection) can be stored at -20°C for long-term storage.

Gaussia Luciferase Assay Protocol (GAR-1)

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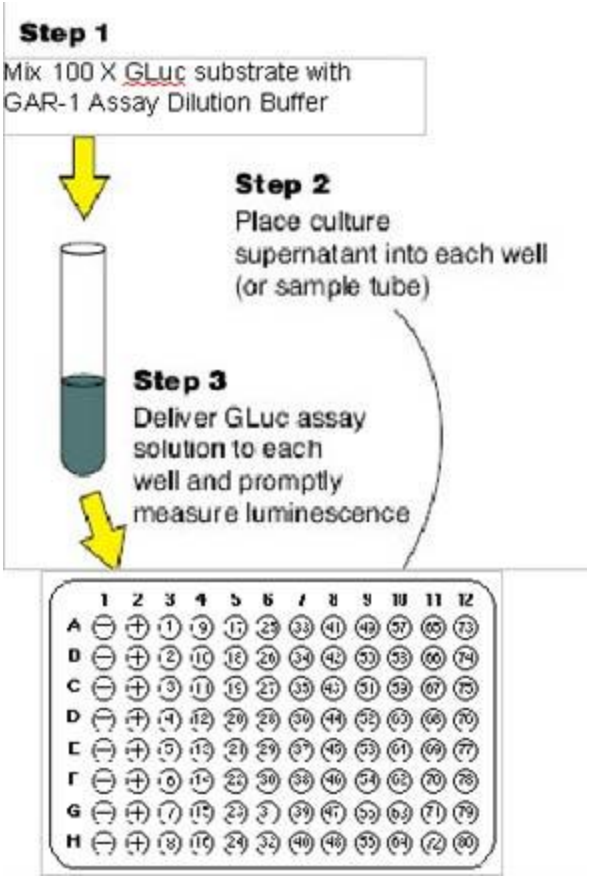

Assay Protocol: Make sure all buffers are at room temperature prior to assay

1. Dilute the concentrated coelenterazine (GAR substrate), provided as a 100X formulation to 1X using the GAR assay dilution buffer. For example to prepare 5 ml of assay reagent dilute 50 ul of 100 X coelenterazine with 4.95 ml of GAR assay dilution buffer.
2. Pipette 20µl of Gaussia Luciferase Sample into assay dish or luminometer tube
3. Add 50 µl of Gaussia luciferase assay reagent (prepared as described in step 1). Mix well
4. 1.Assay in luminometer, integrate for 10 seconds (or as desired)

Intracellular Gaussia luciferase activity

NOTE: If you need to measure intracellular luciferase activity, lyse cells first using the cell-lysis buffer from Targeting Systems. (catalog no 5X CLR-01). Follow cell lysis protocol supplied with the product (see below). Assay as above using 5 ul to 10 ul of lysate

1. Dilute the 5X CLR buffer 1:5 with water.
2. Aspirate cell culture media and wash cells twice with serum free DMEM.
3. Add enough of 1X cell lysis buffer to cover cells. Add enough lysis buffer to cover cell.s (50 ul for 96-well, 300 ul for a 12-well, 800 ul for a 6-well dish and 3 mll for a 10 cm dish
4. Shake for 20 min at 400 rpm on an orbital shaker (room temperature).
5. Mix 5-20 µl of luciferase containing sample or cell lysate with 50 µl of the luciferase assay kit (GAR-1) and read immediately in the luminometer.
6. All assay reagents should be close to room temperature at the time of assay.

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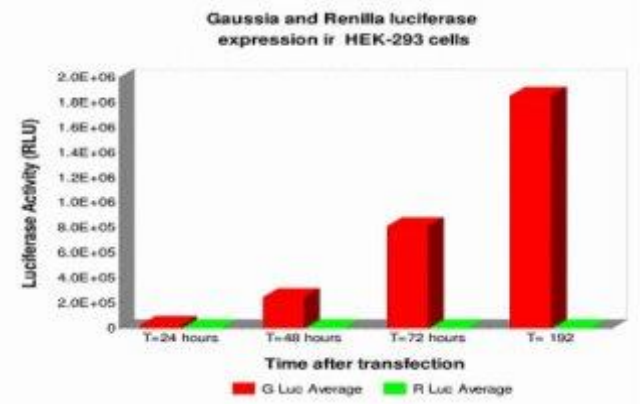
References:

1. Tannous, B.A., Kim, D.E., Fernandez, J.L., Weissleder, R., and Breakefield, X.O. (2005) Mol. Ther, 11, 435-443.
2. Wu, C., Suzuki-Ogoh, C. and Ohmiya, Y (2007) BioTechniques, 42, 290-292.

Custom Reagents:
We can provide custom formulations to fit your HTS application. Call our tech support team at 1-866-620-4018 or email us info@targetingsystems.com or targetingsystems@gmail.com

Please check out our website www.targetingsystems.net for novel luciferase – based multiplexed assays which enable analysis of up to four promoter activities in the same group of transfected cells.



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

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gene expression over a longer time period.