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Gaussia Luciferase Assay reagent (GAR-2B)

Catalog no.	Size	Description	Price
GAR-2B	1000 assays	Gaussia luciferase assay reagent	$420

*Call for special pricing on bulk purchases

Description:

The GAR-2B *Gaussia* Luciferase Assay Kit contains the reagents necessary for assaying *Gaussia* Luciferase (GLuc) activity, most commonly from cell culture supernatants. *Gaussia* Luciferase is a reporter luciferase from the marine copepod *Gaussia* princeps (1,2). *Gaussia* Luciferase can be expressed in mammalian cells using reporter plasmids available from NEB (Refer to the Companion Products). This luciferase, which does not require ATP, catalyzes the oxidation of the substrate coelenterazine in a reaction that produces light (Figure 1), and has several advantages over other luminescent reporter genes.

This kit includes an additional stabilizer component, which allows the use of the assay in high throughput format or without the requirement of an injector-equipped luminometer**.** This three-component assay system provides the user with 2 options: (a) use the assay without stabilizer for enhanced light output or (b) use with the desired amount of stabilizer for enhanced signal stability. The stabilizer component allows the use of the assay in high throughput format or without the requirement of an injector-luminometer. For standard assays giving the highest activity, the kit can be used with the GLuc substrate mixed in the assay buffer. With the stabilized assay protocols, the light emission decays slowly with a half-life of approximately 25 minutes. The addition of stabilizer decreases the absolute value of light output but confers signal stability over time (Fig 2).

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 sample can be used for the assay. Although most of the GLuc is secreted, the high sensitivity of GLuc allows measurements from the cellular fraction.

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

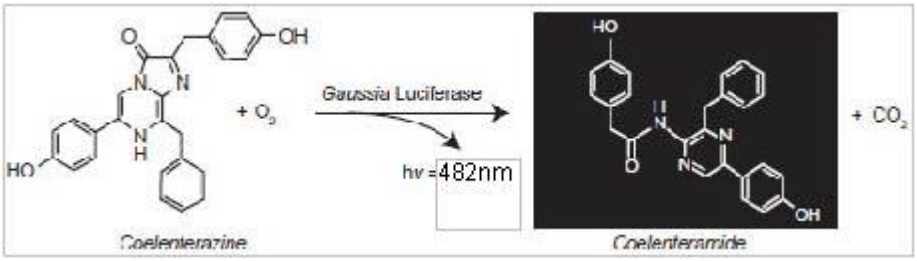

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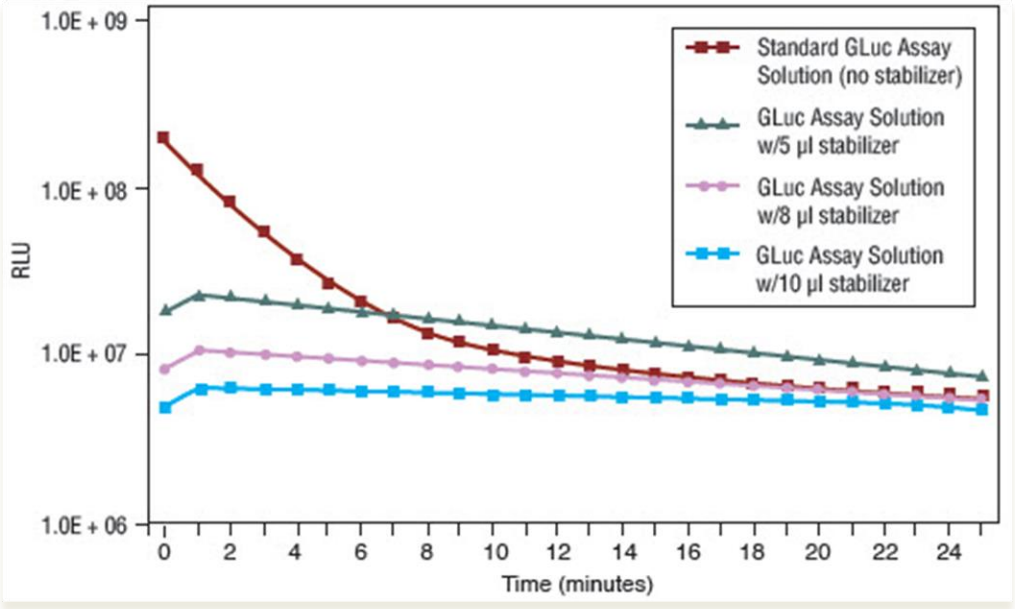
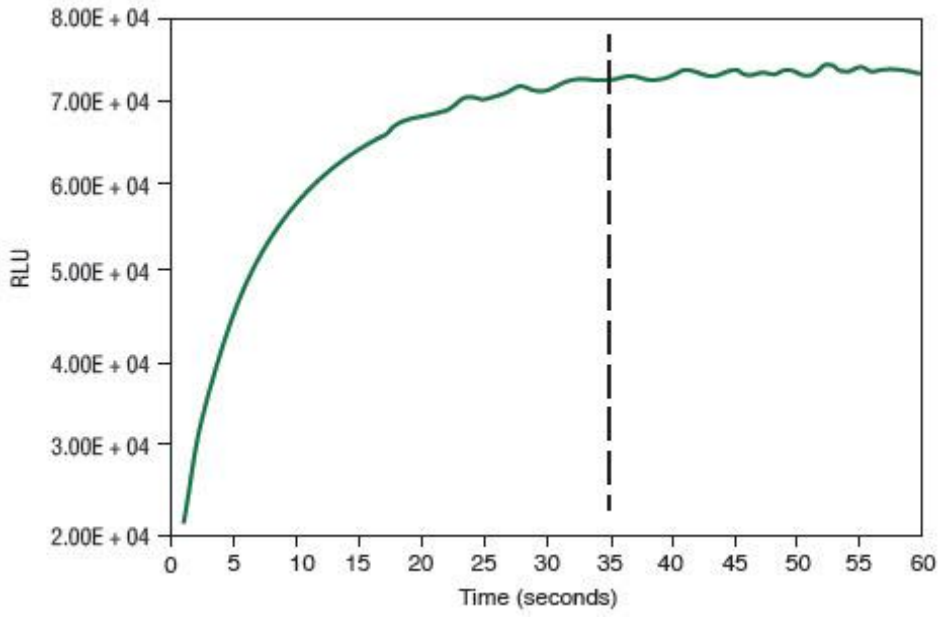
Figure 2: GLuc kinetics using the GAR-2B Assay Kit in either standard or stabilized assay
(ASSAY OF NATIVE GAUSSIA LUCIFERASE). FOR ASSAYING THE MORE STABLE GAUSSIA LUCIFERASE MUTANTS PLEASE USE THE GAR_2B REAGENT WITHOUT STABILIZER



Assays were setup using assay solution without stabilizer or with the indicated amounts of stabilizer

Figure3: : *Gaussia* Luciferase activity after adding GLuc assay solution containing stabilizer to a sample.



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Kit Components

The following **reagents** are supplied with this product:

	Store at (°C)	Concentration	
Gaussia Luciferase Assay Buffer	4	1X	
Gaussia Luciferase Substrate	-20	100X	
Gaussia Luciferase Stabilizer	4	100X	

Advantages and Features

- *Gaussia* Luciferase (GLuc) possesses a natural secretory signal and upon expression is secreted into the cell medium. Therefore, lysing cells in order to assay GLuc activity is not necessary. As a result, GLuc is an ideal reporter gene for time course studies (3).
- GLuc generates over 1000-fold higher bioluminescent signal intensity, when compared to Firefly and Renilla luciferases, making it an ideal transcriptional reporter (3).
- GLuc show the highest reported activity of any characterized luciferases (4).
- The secreted protein is thermally stable (Figure 3) and has extremely high activity in light production for very sensitive assays (2).
- The secreted GLuc is also very stable in the presence of 55µM ß-mercaptoethanol, which is typically used in culturing mouse stem cells .
- The GLuc-containing samples, i.e., growth media or cell lysates, can be stored at -20˚C for long-term storage or 4˚C for several days without loss of activity.
- The stabilizer component of the this assay system provides steady kinetics over a long period of time allowing users the time required for high throughput analysis as well as manually delivered assays.

Notes:

Because of the stability of GLuc, the activity measured in the growth media of GLuc-expressing culture reflects the protein that has accumulated up to the time of sampling.

For the standard assay solution, i.e. solution that does not contain stabilizer, equilibration of the assay solution is not necessary.After adding the GLuc assay solution to the sample, we recommend a delay of 1-5 seconds before taking a measurement. Keeping the delay time consistent across experiments will ensure reproducibility.

For the stabilized assay solution, i.e., the stabilizer-containing GLuc assay solution, the solution should be equilibrated at room temperature for 25 minutes (protect from light in a tightly capped tube/bottle) before adding to the sample.

After adding the equilibrated GLuc assay solution to the sample, we recommend a delay time of 35-40 seconds before taking a measurement in order to reach maximum level of detection. This is especially important when the GLuc activity level is low (e.g. < e4 RLU). For example, the readout obtained after 35-40 seconds of delay is

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~e4; when compare to 30, 20 and 10 seconds of delay, the readouts are as follows: ~2% decrease (for 30 seconds of delay), ~7% decrease (for 20 seconds of delay), & ~20% decrease (for 10 seconds of delay) in RLU (refer to Figure 5).

Use the prepared GLuc assay solution within 2 hours.

The linear range of the luminometer used for the assay must be established. This is easily done by assaying serial dilutions of a sample. In addition, the assay solution itself, as well as the conditioned media (i.e. growth media from untransfected cells) should be included in the assay to establish the background signal in the assay.

If excess activity for the instrument range is found, the sample should be diluted in either PBS or 10% serum-containing media. The integration time can also be reduced.

When assaying the serial dilution of a sample, it is best to assay the most diluted samples first and the most concentrated samples last. This will help to minimize false readings, i.e., cross-talk effect (signals from samples of high RLU cross into that of the next sample). The cross-talk effect seems to be more pronounced when plates (white or black) with clear-bottoms are used.

The Gaussia Luciferase Assay Buffer and the Gaussia luciferase Stabilizer can be stored at 4˚C while the GLuc Substrate must be stored at -20˚C.

References:

1. Verhaegen M. and Christopoulos T.K. (2002). *Anal. Chem*. 74, 4378-4385.
2. Tannous, B.A., Kim, D.E., Fernandez, J.L., Weissleder, R., and Breakefield, X.O. (2005). *Mol. Ther*. 11, 435-443.
3. Wu, C., Suzuki-Ogoh, C. and Ohmiya, Y. (2007). *BioTechniques*. 42, 290-292.
4. Goerke, A., Loening, A., et al. (2008). *Metabolic Engineering*. 10, 187–200.

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ASSAY PROTOCOLS:

NOTE: Most of our customers DO NOT USE THE GAR-2B STABILIZER (included as an OPTIONAL reagent) as the signal without the stabilizer is much higher and the stability is acceptable for most applications. If you are a new user, we recommend you test it WITHOUT the stabilizer first and then use the stabilizer component only if you are working with native Gaussia luciferase and need extended stability

Standard Assay Protocol I (Luminometers without injectors)

1. Prepare the GLuc assay solution (e.g. 100 samples) by adding 50 µl of the 100X GAR Substrate to 5 ml of GLuc Assay Buffer immediately before performing the assay.
2. Mix well by inverting the tube several times (Do not vortex).
3. Set the luminometer for 2–10 seconds of integration.
4. Pipet samples* (5–20 µl per well) into a 96-well white (opaque) or black plate, or a luminometer tube.
5. Add the GLuc assay solution (50 µl) to a sample (i.e. Add the assay solution to only one sample at a time) and promptly measure the luminescence.
6. Repeat Step 5 for all samples.

Standard Assay Protocol II (Luminometers with injectors))

1. Prepare the GLuc assay solution (e.g. 100 samples) by adding 50 µl of100X GAc Substrate to 5 ml of GLuc Assay Buffer immediately before performing the assay. (Be sure to prepare enough assay solution as needed for all samples as well as for priming a particular luminometer as recommended by the manufacturer).
2. Mix well by inverting the tube several times (Do not vortex).
3. Set the luminometer with the following parameters: 50 µl of injection volume and 2–10 seconds of signal integration.
4. Pipet samples* (5–20 µl per well) into a 96-well white (opaque) or black plate, or a luminometer tube.
5. Prime the injector with the GLuc assay solution and proceed with the measurement.

Stabilized Assay Protocol I (Luminometers without injectors)

1. Prepare the GLuc assay solution (e.g. 100 samples) by adding 50 µl of 100X GAR Substrate and 800 µl of GLuc Stabilizer to 5 ml of GLuc Assay Buffer.
2. Mix well by inverting the tube several times (Do not vortex).
3. Incubate at room temperature for 25 minutes (protect from light in a tightly capped tube/bottle) before adding to the sample.
4. Set the luminometer for 2-10 seconds of integration.
5. Pipet samples* (5-20 µl per well) into a 96-well plate (opaque, white or black) or a tube.
6. Add the assay solution (50 µl per well) to all samples.
7. Incubate at room temperature for 35-40 seconds (refer to Notes) and proceed with the measurement.

Stabilized Assay Protocol II (Injector-equipped luminometers)

1. Prepare the GLuc assay solution (e.g. 100 samples) by adding 50 µl of 100X GAR Substrate and 800 µl of GLuc Stabilizer to 5 ml of GLuc Assay Buffer (Be sure to prepare enough assay solution as needed for all samples as well as for priming a particular luminometer as recommended by the manufacturer).
2. Mix well by inverting the tube several times (Do not vortex).
3. Incubate at room temperature for 25 minutes (protect from light in a tightly capped tube/bottle) before adding to the sample.

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4. Set the luminometer with following parameters: 50 µl of injection, 35-40 seconds of delay (refer to Notes), & 2-10 seconds of integration.
5. Pipet samples* (5-20 µl per well) into a 96-well plate (opaque, white or black) or a tube.
6. Prime the injector with the assay solution and proceed with the measurement.

* Approximately 90% of GLuc is secreted out into the growth media after transfection and thus, the GLuc activity is typically assayed from the supernatant (i.e. growth media of GLuc-transfected cells). However, as long as the cells are alive, approximately 10% of GLuc is present inside the cells. Therefore, GLuc activity can also be assayed from the cell lysate. We recommend that the cell lysates be prepared by using Luciferase Cell Lysis Buffer (5X CLR-01), since this lysis buffer is designed to be compatible with *Gaussia*, Cypridina and *Renilla*, Firefly luciferase

Measurement of intracellular Gaussia luciferase activity:
Lyse cells using our lysis buffer (Catalog no 5X CLR-01). Assay as above using 5 ul to 10 ul of lysate
Protocol to measure intracellular luciferase activit:
Lyse cells first using the cell-lysis buffer from Targeting Systems. (catalog no 5X CLR-01):

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 GAR-2B reagent 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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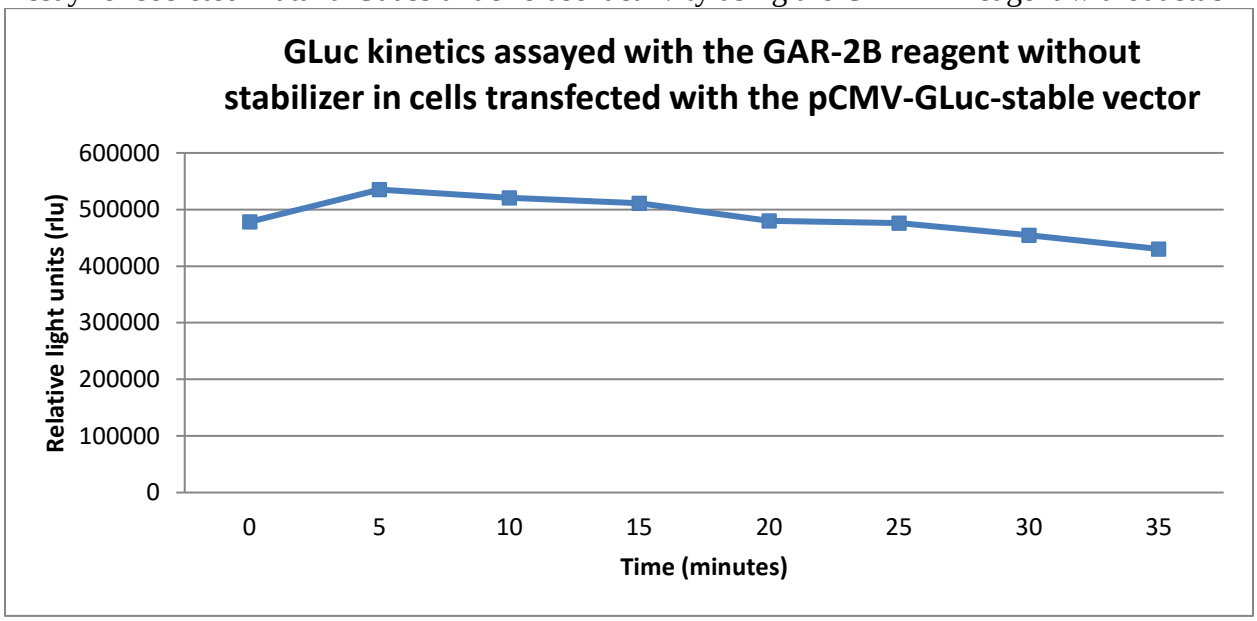
GAR-2B reagent without stabilizers is very useful for measuring measuring mutantmore stable Gaussia luciferase.

NOTE: Targeting Systems offers an expression vector pCMV-GLuc-Stable which expresses a mutant Gaussia luciferase with 2 amino acid replacements. The mutant luciferase is more stable compared to native Gaussia luciferase and can be assayed using the GAR-2B reagent without any stabilizer. The signal intensity is comparable to that of native Gaussia luciferase but as can besseen from the graph below the signal stability is excellent without the need for any added stabilizers.

Assay for secreted mutant Gaussia luciferase actrivity using the GAR-2B reagent without stabilizer:



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