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LiveResponse Luciferase Assay System

Components, storage conditions and protocols

Catalog # LVR-001

1. **pBasic RedFluc** (intracellular form of firefly luciferase) or **pBasic-SRedFLuc** (expresses secreted firefly luciferase, 20 ug) - red firefly luciferase expression vector
2. **pBasic-Gluc (20 ug)**-------Gaussia lcuiferase expression vector
3. **pBasic—Vluc (20 ug)**-----Cypridina (Vargula) luciferase expression vector
4. **pBasicBrenLuc (20 ug)**----Blue-shifted Renilla luciferase expression vector
5. **pBasicGrRenLuc (20 ug)**---Green-emtting Renilla luciferase expression vector

Note: (You may also choose to order vectors expressing the above luciferases under control of the CMV promoter as an alternative to ordering the promoterless vectors)

Assay Reagents:

Gaussia Luciferase Assay Reagent (500 reacns)
Renilla luciferase assay reagent (500 reactions)
(Cypridina) Vargula Luciferase Assay Reagent (500 reacns)
Firefly Luciferase Assay Reagent (500 reactions)

All plasmid vectors and assay reagents should be stored at – 20 ^{o}C with the exception of the Vargulin substrate which we recommend storing at – 80 ^{o}C

Note: The Assay buffers for the luciferase assay reagents can be stored at 4 o C if desired. The tubes containing the luciferase substrates coelenterazine and Cypordiina luciferin (Vargulin) must be tightly capped to prevent oxidation. The Firefly luciferase assay reagent should be kept frozen at -20 ^{o}C.

Protocols: The assay protocols for the 4 luciferase components of the LiveResponse System are provided below: Note that each of the following luciferase assay reagents contains enough reagent for 500 assays in microtiter wells or luminometer tubes.

FLAR-1: Assay reagent for measurement of Firefly Luciferase.

RLAR-1: Assay reagent for measurement of Renilla luciferase.

GAR-3: Assay reagent for measurement of Gaussia luciferase.

VLAR-1: Assay reagent for measurement of Cypridina (Vargula) luciferase.

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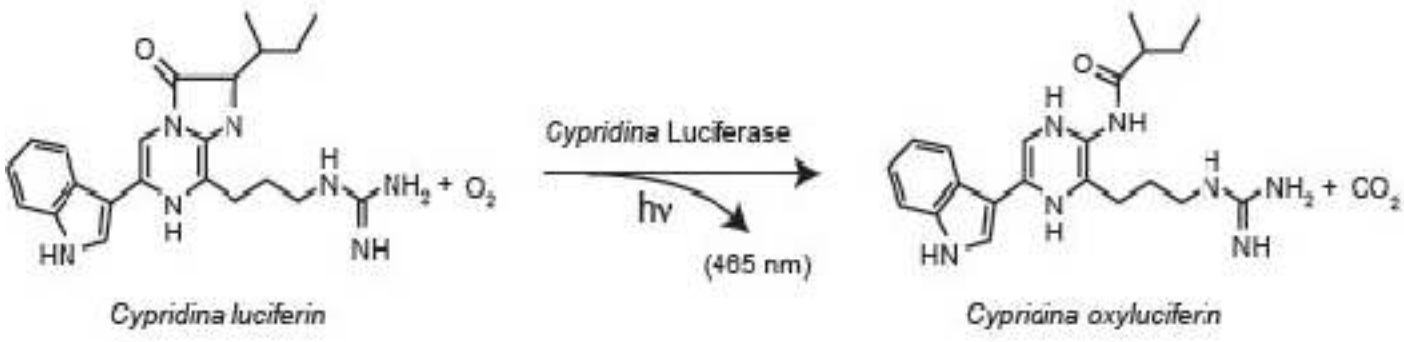
VLAR-1: Assay reagent for Cypridina (Vargula) Luciferase

Product Description

Cypridina (Vargula) luciferase: Cypridina luciferase, (formerly known as Vargula luciferase) from the marine ostracod Vargula Hilgendorfi is a secreted luciferase with an emission max of 460 nm. It is one of the brightest known luciferases with the highest turnover number. Unlike Firefly luciferase, Cypridina lcufierase does nto require ATP and catalyses oxidation of it´s unique substrate Cypridina luciferin in a photochemical reaction described below (Figure 1).



Cypridina Luciferin is different form colenterazine, the substrate for Renilla , Gaussia and Metridia luciferses. On account of it´s unique substrate and bright, secreted luciferase activity Cypridina Luciferase is particularly useful in multiplexed assays involveing Gaussia , Renilla or Firefly luciferses.. Secreted CLuc is a very stable protein. Because of this property, the activity measured from the supernatant reflects the amount of protein accumulated up to the time of sampling. Multiple samples can therefore be obtained from the same transfected cells

Cypridina luciferase is naturally secreted form cells (Figure 2) . Therefore cell lysis is not necessary for measurement of luciferase activity.

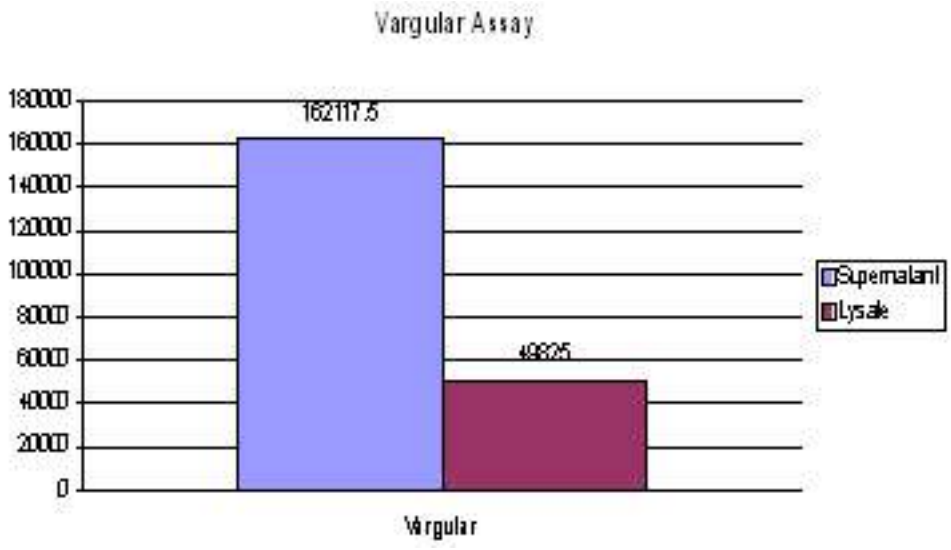


Figure 2: Intracellular and secreted Cypridina luciferase activity

Luciferase activity in cell supernatants and cell lysates of cell transfected with either a plasmid vector expressing secreted vargula luicferase. In cells transfected with the secreted form of modified vargula luciferase, 80% of the activity is sereted into the cell supernatant and only 20% is cell–associated.

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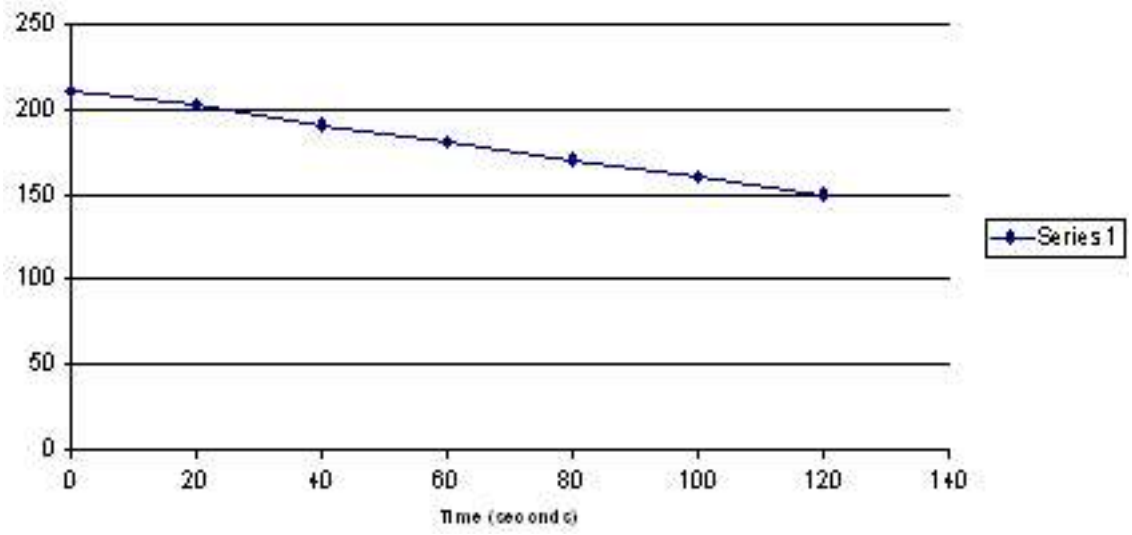

Figure 3: Kinetics of light emission. The stability of the bioluminescent signal of Cypridina Luciferase was assessed using supernatants from HEK 293 cells transiently transfected with the pCMV-VLuc expressionvector.

Cypridina Luciferase Assay Protocol:

Contents and Storage:

Each kit contains the following:

1. Cypridina luciferin substrate (100 X) Store at – 80 ° C.
2. Cypridina substrate dilution buffer (20 ml) (Provided in a brown bottle) This can be stored at 4 ° C.
3. CLAR (Cypridina luciferase assay buffer). The VLAR buffer (Cypridina luciferase assay buffer) is provided in a 50ml bottle. This can be stored at 4 ° C .

Protect the Cypridina substrate and diluted substrate solution from light. Avoid leaving tubes open for long.

Stability of the undiluted 100X Cypridina substrate is guaranteed for 1 year from the date of purchase. The substrate once diluted should be stored at – 80 ° C and used within 3 months.

Preparation of 1X Cypridina substrate and Cypridina assay solution

Preparation of 1X Cypridina substrate solution:

Dilute the 100X Cypridina luciferase substrate with the appropriate amount of Cypridina substrate dilution buffer. The Assay protocol uses 20 ul of the 1X Cypridina substrate to assay each sample so to assay a 100 samples you would mix 200 ul of the 100X Cypridina substrate with 1.8 ml of the Cypridina substrate dilution buffer.

Preparation of the working Cypridina luciferase assay solution:

Thaw the VLAR buffer completely at room temperature just before use. Each assay reaction uses a mixture of 20 ul of the Cypridina substrate with 40 ul of the VLAR buffer (Cypridina luciferase assay buffer) . For instance if you need to assay 100 samples you should mix 2 ml of 1X Cypridina substrate solution with 3.5 ml of VLAR buffer just before use. Make sure both solutions are thawed to room temperature prior to mixing.

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Note: Cypridina luciferase is a secreted enzyme and most of the activity is secreted (75-80 percent) is in the supernatant. We recommend using OptiMEM 1 or complete media with low serum content (3 percent or less) as this reduces the background of the assay. We recommend assaying samples at 48 hrs post transfection. In order to measure Cypridina luciferase activity in the Cell lysates we recommend using the 5X Cell Lysis Reagent (catalog no. 5X CLR2, see protocol below) from Targeting Systems, as this is compatible for assaying all luciferases (Cypridina luciferase, Renilla luciferase, Gaussia luciferase, Firefly luciferase as well as Beta galactosidase) in the lysate

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)

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 cells (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 100 µl of the luciferase assay kit (TS-1) and read immediately in the luminometer.
6.All assay reagents should be close to room temperature at the time of assay.

Assay Protocol:

Luminometer without injectors:

Pipette out 5 to 20 ul of Cypridina luciferase sample (supernatant or cell lysate) into each well of a 96-well dish. You may use white (opaque) or black plates or cuvettes.

Add 55 ul of the working Cypridina luciferase assay solution (prepared as described above) to each well or cuvette. Mix well and read in the luminometer set for a 2-10 second integration (this can be varied if desired)

Luminometer with injectors:

Note: Be sure to prepare enough of the working Cypridina luciferase assay solution (prepared as described above) as needed for all samples as well as for priming the injector as suggested by the manufacturer. Protect this solution from light.

Set the luminometer with the following parameters: 55 µl injection, 1–2 seconds of delay and 2–10 seconds of integration.

Pipet samples (5–20 µl per well) into a 96-well white (opaque) or black plate or cuvettes.

Prime the injector with the Cypridina luciferase assay solution and proceed with measurements.

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. Please check out our webstie 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.

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Relevant References:

1. Thompson, E. M., Nagata, S., and Tsuji, F. I. (1990) Vargula hilgendorfii luciferase: a secreted reporter enzyme for monitoring gene expression in mammalian cells Gene (Amst.) 96, 257–262

2. Shin-ya Nishide, Sato Honma, Yoshihiro Nakajima, Masaaki Ikeda, Kenkichi Baba, Yoshihiro Ohmiya, and Ken-ichi Honma (2006) New reporter system for Per1 and Bmal1 expressions revealed self-sustained circadian rhythms in peripheral tissues. Genes Cells, Oct 2006; 11: 1173 - 1182.

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Firefly Luciferase Assay reagent (FLAR-1)

Brightest Signal Intensity, best signal stability - For HTS applications

Product Description

The luciferase assay kit FLAR-1 is a homogenous luciferase gene detection system. Targeting Systems has developed this FLAR-1 luciferase assay kit to facilitate measurement of luciferase activity in HTS (high throughput applications). The kit supplied by Targeting Systems are one step assay kit, i.e. the reagent has all the ingredients necessary for lysing cells as well as the luciferin substrate and stabilizers of the luciferase reaction in a single solution unlike other kits where the assay buffer has to be mixed with the luciferin substrate provided in a separate bottle. The FLAR-1 luciferase assay kit can be used to measure luciferase activity in pre-lysed cell extracts or it can be added directly to the cells (see attached protocol sheet). The assay is compatible with both sample processing robots and with reagent injectors in many luminometers

Note: The FLAR-1 reagent can be used to measure firely luciferase activity in both supernatants or lyses of cells transfected with expression vectors expressing firefly luciferase either as an intracellular or secreted form.

Principle of the Luciferase Assay

The luciferase assay kit is based upon the bioluminescent measurement of firefly luciferase. This enzyme catalysis the formation of light from ATP and luciferin according to the following reaction:

The intensity of light emission is linearly related to the amount of luciferase and is measured using a luminometer. Luciferase is the most widely used genetic reporter in studies on gene expression due to it's high sensitivity dynamic range and its natural absence from mammalian cells. The luciferase assay kit offered by Targeting Systems offers the advantages of high sensitivity, consistent reproducibility and cost effectiveness along with the added convenience of a one step assay. The FLAR-1 luciferase assay reagent has been formulated with the cell lysis components, the luciferrin substrate and the bioluminescence stabilizers all included in a single assay reagent.

Specifications:

Limit of detection – less than 1 fg of luciferase per sample Luciferase assay kit (FLAR-1): This kit can be conveniently used for measuring luciferase activity in 96-well plates (or in preparations of lysed cells). As seen in the figure below, the bioluminescent signal is quite stable with a half life greater than 60 mins. The FLAR-1 luciferase assay kit can also be used to measure luciferase activity in prelysed cell extracts. In this case 5-50 µl of the cell lysate is mixed with 100 ul of the luciferase FLAR-1 assay reagent, mixed well and read immediately in the luminometer. The FLAR-1 reagent includes several stabilizers of the luciferase enzyme in its composition. Since the stabilizers used are not luciferase inhibitors a stable bioluminescent signal

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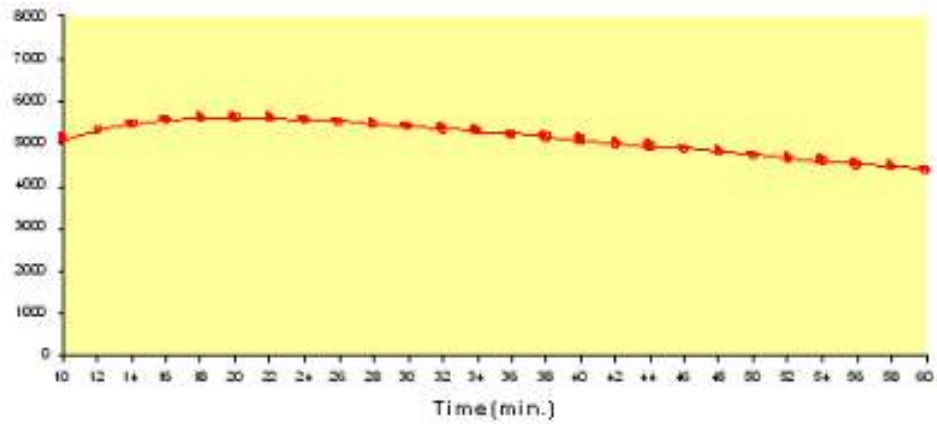

Stability of the bioluminescent signal using the FLAR luciferase assay reagent). Results are expresses as the mean of quadruplicate determinations (CV less than 5%). The intensity of the bioluminescent signal using the FLAR reagent from Targeting Systems is comparable to the intensity of the bioluminescent signal in assays using Promega's Bright glo reagent.

In the experiment shown above the FLAR reagent was directly added to the supernatant cell culture media.

Protocol:

Assay In 96-well Format:
Add 100 µl of the FLAR-1 reagent to 50 µl or 100 µl of the supernatant culture media from cells grown in multi-well tissue culture dishes. Incubate for at least 10 minutes and read in a lumionometer or micro plate reader.

Assay Of Luciferase Activity In Cell Lysates: The FLAR-1 luciferase assay reagents can also be used to measure luciferase activity in pre-lysed cells. Mix 5-50 µl of luciferase containing sample or cell lysate with 100 µl of the luciferase assay kit (FLAR-1) and read immediately in the luminometer. All assay reagents should be close to room temperature at the time of assay. NOTE: If you need to lyse cells first use the cell lysis buffer from Targeting Systems or the Cell culture lysis reagent from Promega (E1531).

Intracellular Firefly luciferase activityLyse cells using our lysis buffer (Catalog no 5X CLR-01). Follow cell lysis protocol supplied with the product. Assay as above using 5 ul to 10 ul of lysate

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) for novel luciferase – based multiplexed assays which enable analysis of up to four promoter activities in the same

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, 300ul 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 100 µl of the luciferase assay kit (FLAR-1) and read immediately in the luminometer. min at 400 rpm on an orbital shaker (room temperature).
6. All assay reagents should be close to room temperature at the time of assay.

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Gaussia Luciferase Assay reagent (GAR-2, Catalog ·0090A)

Stable version

Product Description

The GAR-2Assay Kit includes an additional stabilizer component, as well as a modified assay buffer, which allows the use of the assay in high throughput format. With the standard protocol the light emission decays rapidly. The addition of stabilizer decreases the absolute value of light output slightly but confers excellent signal stability (approximately 10% decay in 1 hour, Figure 2A). 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 stability.. Although the Gaussia luciferase signal obtained upon assaing samples with GAR-2 shows excellent stability making it suitable for HTS, we have recently developed a further improvement (GAR-2B)wherein the signal intentiy is much brighter than that obtained using the GAR-2 reagent and the signal stability is also much better.

About Gaussia Luciferase:

Gaussia Luciferase 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 iaddition 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

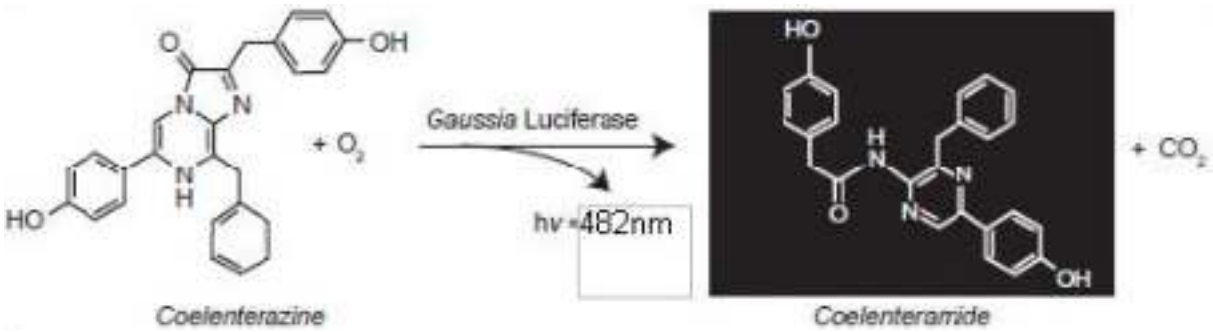

Figure 2: Stability of the GLuc bioluminescent signal using the GAR-2B reagent. Panel A: With Stabilizer. Panel B: Without Stabilizer Using the GAR-2B version of the Gaussia luciferase assay reagent the bioluminescent signal remainsvery stable (Panel A). This reagent is useful for HTS applications in which a large number of samples need to be assayed. In the absence of the stabilizer, the signal.intensity is a little higher initially but decays faster than in the presence of stabilizer. **Note:** Data presented is average of triplicate determinations measured on a Turner TD2020

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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).
- The stabilizer component of this assay system provides steady kinetics over a longer time period allowing users the time required for high-throughput analysis as well as manually delivered assays.
- 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 secreted GLuc is also very stable in the presence of 55 µM β-mercaptoethanol, which is typically used in culturing mouse stem cells.
- The GLuc-containing samples (i.e. growth media or cell lysates after transfection) can be stored at -20°C for long-term storage.luminometer.

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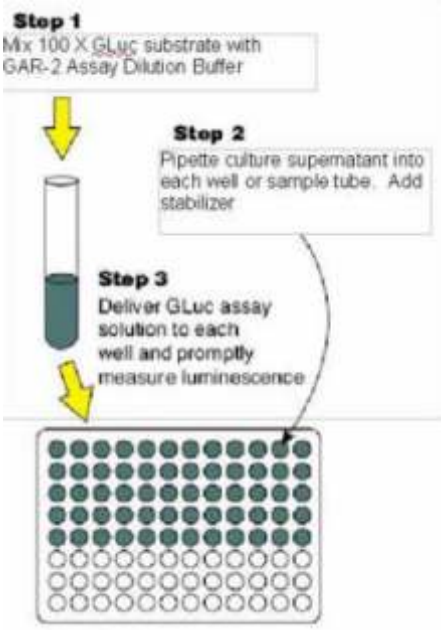
Gaussia Luciferase Assay Protocol (GAR-2)



Kit Contents:

- 1.50ml GAR- (Gaussia Assay reagent) Dilution Buffer Store at 4°C
- 8ml GAR Stabilizer- Store at 4 °C
- 500µl Coelenterazine substrate (100X), Store tightly capped at -20°C

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 in Assay dish/tube
3. Add 4 ul or 8 µl GAR stabilizer and also do one assay without stabilizer
4. Add 50 µl of Gaussia luciferase assay reagent (prepared as described in step 1)
5. Assay in luminometer integrate for 10 seconds (or as you wish)

Intracellular Gaussia luciferase activity

Lyse cells using our lysis buffer (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

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)

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 100 µl of the luciferase assay kit (TS-1) and read immediately in the luminometer.
6. All assay reagents should be close to room temperature at the time of assay.

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.

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Renilla Luciferase Assay reagent (RLAR-1)

Stable version

Description:

The RLAR-1 Assay Kit includes an additional stabilizer component, as well as a modified assay buffer, which allows the use of the assay in high throughput format. With the standard protocol the light emission decays rapidly. The addition of stabilizer confers excellent signal stability (approximately 10% decay in 1 hour, Figure 2A). 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 stability..

About Green–emitting Renilla Luciferase:

Green-emitting Renilla Luciferase: The emission max of the Green-emitting Renilla luciferase (527 nm) makes it ideal for multiplexed assays with blue and red emitting luciferases. This luciferase has been engirneered for improved brightness (about 40 times brighter than human codon optimized native Renilla Reniformis luciferase) and extended stability of the bioluminescent signal both in vitro and in vivo. Native Renilla luciferase is an intracellular enzyme and does not contain a secretory signal. The Green-emitting mutant has therefore been modified with a synthetic secretory signal at the amino terminal end to express it in a secretable form. The Green-emission maximum of this Renilla mutant offers advantages in multiplexed applications with Blue and Red-emitting luciferases. Promoter acitivty can be assessed by quantitating REnilla luciferase activity in the supernatant media or cell lysates of transfected cells.

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

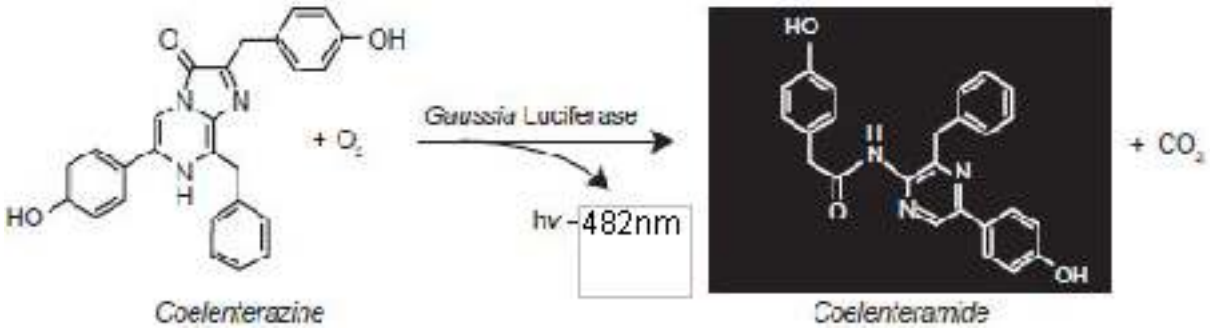

Figure 2: Stability of the Renilla luciferase (RLuc) bioluminescent signal using the RLAR-1 reagent. This reagent is useful for HTS applications in which a large number of samples need to be assayed. In the absence of the stabilizer, the signal.intensity is a decays faster than in the presence of stabilizer. Note: Data presented is average of triplicate determinations measured on a Turner TD2020 luminometer

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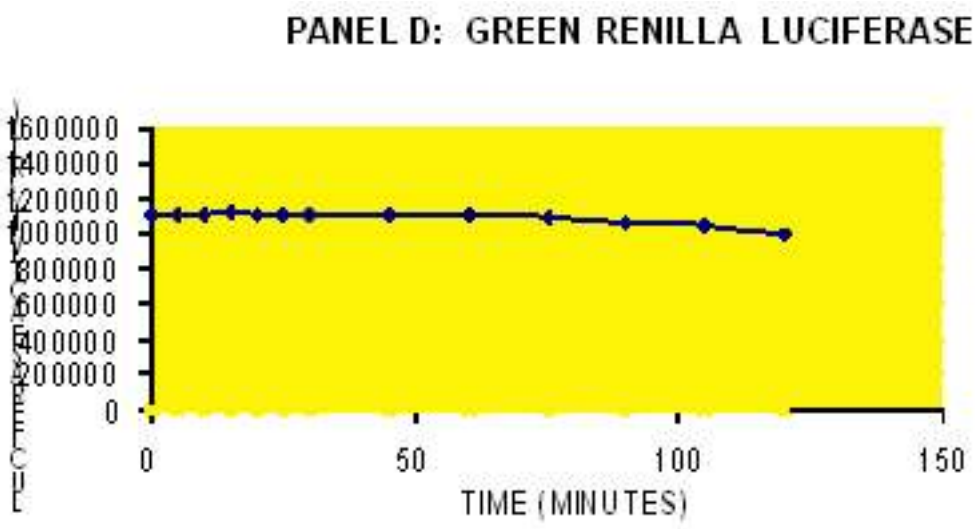
Kinetics of Renilla Luciferase Activity



Advantages:

• Native Renilla Luciferase does not possess a natural secretory signal, However, the green=emitting renilla mutant has been engineered with a secretory signal and upon expression is efficiently secreted into the cell medium. Cell-lysis is therefore not necessary for assaying the luciiferase.

• The Green–emitting Renilla Luciferase is about 40 times brighter than commonly used Firefly and Renilla Luciferases (human codon optimized) when expressed in mammalian cells under the same promtoter, making it an ideal transcriptional reporter (1).

• The GLuc-containing samples (i.e. growth media or cell lysates after transfection) can be stored at -20°C for long-term storage.

• Renilla Luciferase Assay Protocol (RLAR-1)

Kit Components:

1. 50ml RLAR- (Renilla Luciferase Assay reagent) Dilution Buffer. Store at 4°C
2. 4ml RLAR Stabilizer- Store at 4°C
3. 250µl Coelenterazine substrate (100X), Store tightly capped at -20°C

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

1. Dilute the concentrated coelenterazine (RLAR substrate), provided as a 100X formulation to 1X using the RLAR assay dilution buffer. For example to prepare 5 ml of assay reagent dilute 50 ul of 100 X coelenterazine with 4.95 ml of RLAR assay dilution buffer.
2. Pipette 20µl of Gaussia Luciferase Sample in Assay dish/tube
3. Add 4 ul or 8 µl RLAR stabilizer and also do one assay without stabilizer.
4. Add 100 µl of Gaussia luciferase assay reagent (prepared as described in step 1)
5.Assay in luminometer integrate for 10 seconds (or as you wish)

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Intracellular Renilla luciferase activity

Lyse cells using our lysis buffer (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

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)

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 ml 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 100 µl of the luciferase assay kit (TS-1) and read immediately in the luminometer.
6. All assay reagents should be close to room temperature at the time of assay.