

Expression vectors and assay systems for Renilla luciferase

Blue and green-emitting secreted luciferases from Renilla Reniformis with Improved properties and brighter signals

Applications:

- ◆ Improved secreted luciferases with increased brightness and better stability for high throughput screening.
- ◆ Mammalian expression vectors for studying regulation of gene expression
- ◆ Some of the multiplexed assay systems based on the Green-emitting Renilla luciferase permit measurement of two or three different luciferase reporters in the same sample using a single assay reagent by spectrally resolving the luciferase activities of the individual luciferases (each of which uses a different substrate and emits at a distinct wavelength).
- ◆ Green-emitting Renilla luciferase is an excellent reporter for in vivo imaging application and analysis of gene expression in vivo.

The demand for multiplexed reporter systems to draw parallels between multiple parameters within the same cell is ever increasing. Because high throughput and ultra-high throughput screening has become the norm in the Pharmaceutical research facilities, the ability to draw correlations in low volume assays has created a trend towards fully automated miniaturized assays using 384 well and 1536-well formats. These requirements have created a need for assays that require no intervention from the researcher during screening, minimize number of handling steps and have the ability to deliver consistent results in single micro liter volumes.

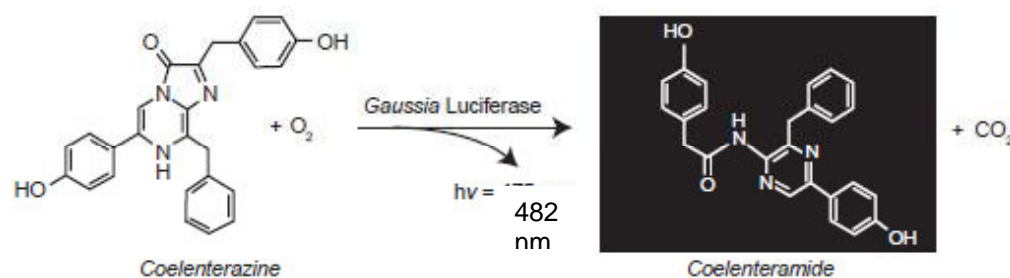
The first requirement for applicability of a luciferase reporter for screening applications is sensitivity (increased brightness), stability of the bioluminescent signal over a long time, and an emission max that would allow multiplexing with other luciferase reporters. Clearly a greater the number of luciferase reporters would allow one to analyze regulation of multiple promoter activities in the same sample of transfected cells without significantly increasing the number of handling steps or assay time. It is to meet these requirements that Targeting Systems has developed a series of novel luciferase reporters with different emission maxima that are suitable for multiplexed assays..

Principle of the Luciferase Assay

Renilla luciferases cloned from the soft coral sea pansy Renilla Reniformis catalyze a bioluminescent reaction that utilizes coelentraxine as a substrate and do not require ATP for their signal, simplifying their use in a number of reporter applications. Mutants of Renilla luciferase with improved properties have been developed for expression as secreted reporters with the goal of establishing a one-step dual luciferase assay wherein a single assay solution can measure relative luciferase activities of two color-shifted variants of Renilla luciferase in the supernatant medium based on spectral resolution. The Green-shifted Renilla mutant (emission max 527 nm) chosen for this purpose is about 100-times brighter than native Renilla, shows improved stability both in vitro and in vivo. The green-shifted variant (emission max of 527 nm), can be conveniently multiplexed with other luciferase reporters like Cypridina luciferase and Firefly luciferase.. Both these variants offer the advantages of being secreted reporters enabling evaluation of gene expression without lysing the cells. Further the use of a single solution for a dual luciferase assay in addition to making the assay far more cost-effective, also greatly reduces the assay time. The most important advantage of using a dual secreted system is that drug responsiveness or regulation of gene expression can be studied at multiple time points from the same group of transfected cells. The increased brightness of the green variant increases sensitivity of the assay and is particularly advantageous for analyzing weak promoters or studying gene expression in hard-to-transfect cells.

The use of secreted luciferases until very recently, has been limited. This However, has changed with the introduction of Targeting Systems' "LiveResponse" Luciferase reporter panel which includes 5 novel secretable luciferases. Gaussia luciferase with an emission max of 482 nm has become a very popular reporter and Vargula (Cypridina) luciferase offered by Targeting Systems although comparable in brightness to Gaussia luciferase has an emission max at 463 nm. Vargula luciferase which uses a different substrate (Vargulin) is an excellent complement (as a third secreted reporter) to the dual Renilla assay. Other luciferases such as the secretable red emitting *Luciola* firefly luciferase will prove advantageous either for use as a denominator reporter or if additional multiplexing is desired.

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



Advantages:

- ◆ Cells expressing the secreted Green-emitting Renilla luciferase mutant show about about 40 times greater luciferase activity than cells expressing native Renilla luciferase (human codon optimized) under identical promoters
- ◆ High Sensitivity. The mutant Renilla luciferase reporters have improved properties (higher signal intensity, stability) compared to native Renilla luciferase.
- ◆ Since both reporters are secreted, gene expression/drug responsiveness can be monitored over time without killing the cells. The bioluminescent signal emitted by the green Renilla mutant is very stable making it useful for HTS.
- ◆ Highly cost-effective, and suitable for high throughput applications. The green emitting Renilla mutant is atleast 50X brighter than native Renilla luciferase making it excellent for ex vivo monitoring of in vivo processes as well as
- ◆ In vivo imaging applications.. The green-emitting Renilla luciferase is particularly well suited for multiplexed applications with Cypridina luciferase and Red emitting *Luciola* luciferase (both offered by Targeting Systems)
- ◆ Several multiplexing options available for dual secreted, dual intracellular or triple luciferase reporter assays.

Products Offered:

- ◆ Renilla luciferase expression vectors
- ◆ Assay reagents for single , dual and triple luciferase reporter assays based on renilla luciferase
- ◆ Lentiviral products based on renilla luciferase for in vivo imaging applications

Kinetics of Renilla Luciferase Activity

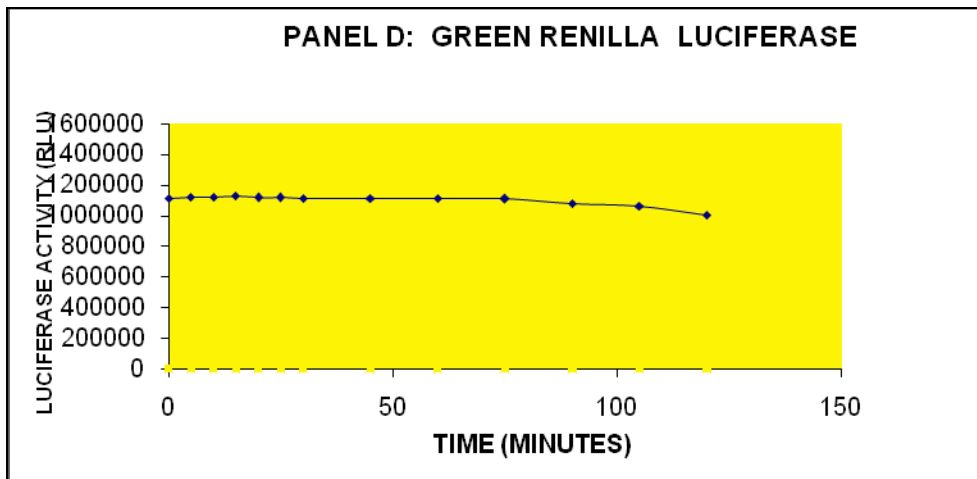
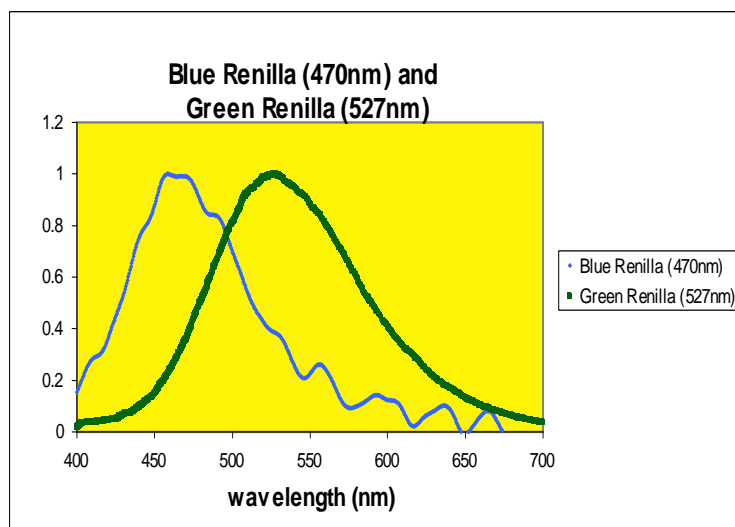


Figure 2A: Stability of the Renilla luciferase (RLuc) bioluminescent signal using the RLAR-1 reagent. This reagent is useful for HTS applications in which a large number of samples need to be assayed. In the absence of the stabilizer, the signal intensity is a decays faster than in the presence of stabilizer.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

Figure 2B: Emission spectra of blue and green-emitting Renilla luciferases



Renilla Luciferase Assay Protocol (RLAR-1)

Kit Components:

1. 100ml RLAR- (Renilla Luciferase Assay reagent) Dilution Buffer
Store at 4 °C
2. 8ml RLAR Stabilizer- Store at 4 °C
3. 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 (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 µl 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 µl 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)

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 µl to 10 µl 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 µl for 96-well, 300 µl for a 12-well, 800 µl 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.

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.

Renilla luciferase-based products

Catalog No.	Size	Description	Price
GRL-003	25µg	pBasic-GRenLuc: Promoterless vector expressing secreted Green Renilla luciferase	\$299.00
GRL-001	25µg	pCMV-GRenLuc: Expresses secreted Green Renilla luciferase under CMV promoter	\$299.00
BRL-003	25µg	pBasic-BIRenLuc: Promoterless vector expressing secreted Blue Renilla luciferase	\$299.00
BRL-001	25µg	pCMV-BIRenLuc: Expresses secreted Blue Renilla luciferase under CMV promoter	\$299.00
		SINGLE ASSAYS	
RLAR-1	1000 assays	Renilla Luciferase Assay Reagent	\$400.00
		DUAL LUCIFERASE ASSAYS	
DLAR-2	1000 assays	Renilla-Red Firefly Luciferase Assay Reagent	\$850.00
DLAR-5	1000 assays	Cypridina-Renilla Luciferase Assay Reagent	\$850.00
		TRIPLE LUCIFERASE ASSAYS	
TLAR-1	1000 assays	Cypridina-Green Renilla-Red Firefly Assay Reagent	\$1000.00
		Lenti-luciferase products based on Renilla luciferase	
LP-09	pLenti-CMV-GrRenLuc	Lentivirus expressing Green secreted Renilla, pLenti-CMV-GrRenLuc plasmid, 1000 assays of the luciferase assay reagent RLAR-1	\$2000
LP-10	pLenti-CMV-GrRenLuc-RFP	Lentivirus expressing Green Renilla luciferase under control of CMV promoter and RFP under phosphoglycerokinase promoter, pLenti-CMV-GrRenLuc-IRES-ORFP plasmid, 1000 assays of the luciferase assay reagent RLAR-1	\$2000
LP-06	pLenti-CMV-GrRenLuc	Control Lenti plasmid expressing Green Renilla luciferase under CMV promoter	\$1650
LP-12	PLenti-CMV-GrRenLuc-RFP	Control Lenti plasmid expressing Green Renilla luciferase under control of CMV promoter and RFP under phosphoglycerokinase promoter	\$1650
Lenti-Custom	Lenti-Custom	Any of the above expressing luciferase under your promoter of interest. (Subject to availability)	\$2500
LP-09	pLenti-CMV-GrRenLuc	Lentivirus expressing Green secreted Renilla, pLenti-CMV-GrRenLuc plasmid, 1000 assays of the luciferase assay reagent RLAR-1	\$2000

Note: Plasmid maps of the renilla luciferase expression vectors can be obtained from our website www.targetingsystems.net

The CMV promoter is covered by U.S. Patents 5,168,062 and 5,385,839 and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA52242.

Commercial users/ For profit companies and foundations will possibly require a license from Targeting Systems and other sources. License for use of the native Renilla reniformis gene has been obtained from Chemicon. (Millipore Corporation, Temecula) Please contact 1-866-620-4018 for more information.

These products are sold for research use only. They cannot be used in humans. The Renilla expression vectors are covered by issued as well as pending patents. Commercial use of these products requires licenses from Targeting Systems. and other sources (Millipore Corporation, Temecula, CA)

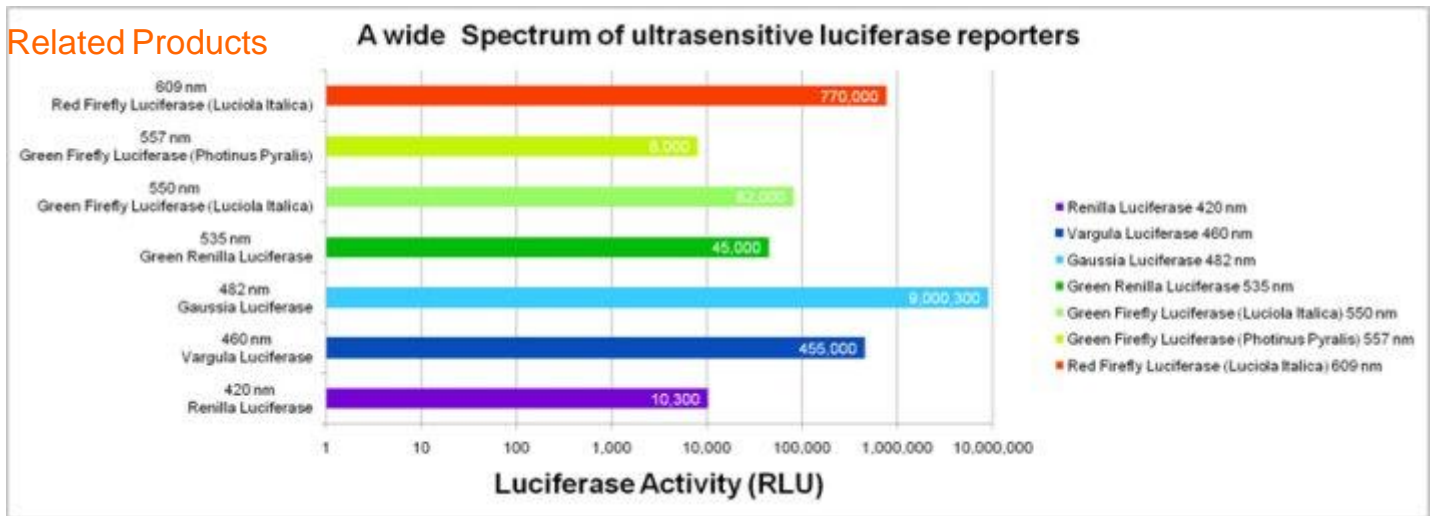


Figure 3: A comparison of the *Luciola Italica* red and green-emitting luciferases with other luciferase reporters. Please note that the *Photinus* luciferase used in this experiment was the PGL3 series *Photinus* luciferase subcloned under control of the CMV promoter. The experiment was conducted in HEK-293 cells using either Gaussia luciferase or Vargula luciferase as the denominator plasmid.

Our newest offering –

LiveResponse, A panel of 5 novel secreted luciferases- The brightest and the best.

Study multiple pathways in living cells by analyzing activities of up to 6 different promoters, each controlling expression of a different secretable reporter. Ideal for ex vivo monitoring of in vivo processes...

Available for the first time as part of our LiveResponse collection- A secretable red-emitting luciferase reporter. The red-emitting luciferase from *Luciola Italica* has been especially engineered and modified to become a secretable reporter which appears very promising both for in vivo imaging applications: and as an additional reporter gene for multiplexing with the dual renilla system.



Multiplexing Options (Dual and Triple luciferase reporter assays):

DLAR-2 :

A dual assay reagent based on a secreted (or intracellular) Green-emitting Renilla luciferase and a secreted (or intracellular) Red-emitting firefly (Luciola) luciferase. This assay is compatible with high throughput applications.

DLAR-5 :

A dual assay reagent based on Cypridina luciferase (secreted) I and a secreted (or intracellular) Green-emitting Renilla luciferase. This assay is compatible with high throughput applications.

TLAR-1 :

A single solution-based triple luciferase reporter assay involving Cypridina luciferase multiplexed with Green-emitting Renilla luciferase and Red-emitting Firefly luciferase. This assay is compatible with high throughput applications. This assay is also optionally available in a format where the three luciferases can be assayed separately using three different assay reagents

Study apoptosis and GPCR profiling while analyzing gene expression:

Instead of doing conventional caspase assay or cell proliferation assays it is now possible to use some components of the LiveResponse system to assess state of transfected cells by simply subcloning the NFκB or CREB upstream of the luciferase gene in the pCMV-Red Fluc vector

Dual Luciferase Reporter Assay based on Renilla luciferase

Green Renilla luciferase, and Red Italicica luciferase; Green Renilla and Cypridina luciferase

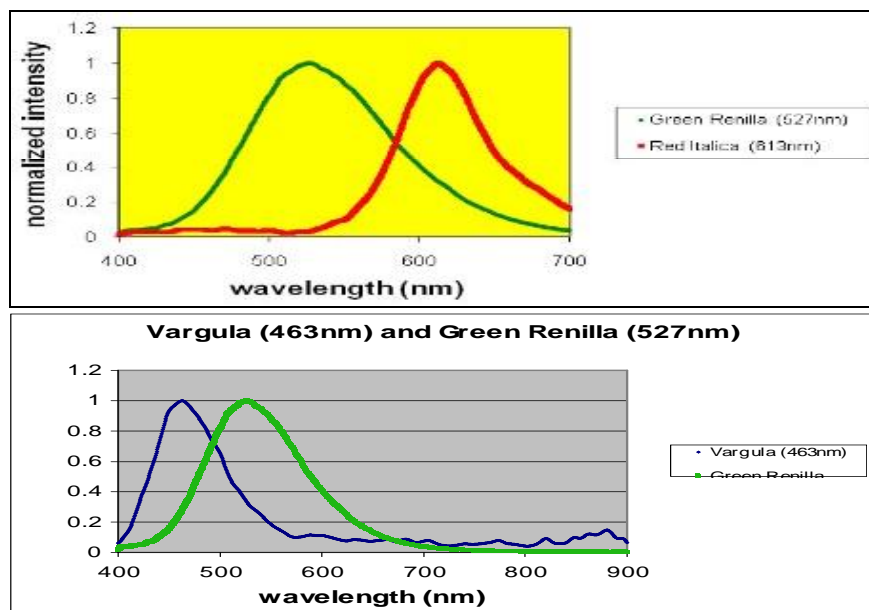


Figure 4: Emission spectra of Green Renilla luciferase and Red Firefly luciferase or Green renilla and Cypridina luciferase in samples of transfected cells (lysates or supernatants). The emission spectra were recorded on a Fluorolog-3 spectrofluorometer (Horiba Scientific, Japan) using a liquid nitrogen cooled CCD. The luciferases were assayed by mixing 200 ul of the sample with the appropriate luciferase assay reagent to obtain spectral profiles. Emission max of Green-emitting Renilla Luciferase is 527 nm; Red italicica is 617 nm. (Data courtesy of Justin Rosenbera. Dr Bruce Branchini's lab. Connecticut Colleea. USA)

Dual luciferase options with Green –emitting renilla luciferase

Red firefly luciferase- Emission max 617 nm

Cypridina Luciferase : emission max 467 nm

Dual assays are offered in two formats: Each luciferase can be measured separately since they use different substrates or they can be assayed in the same sample using a single assay reagent and spectrally resolving the two luciferase activities based on differences in their emission maxima

A triple luciferase reporter assay based on Green renilla, Cypridina luciferase and Red Italic luciferase

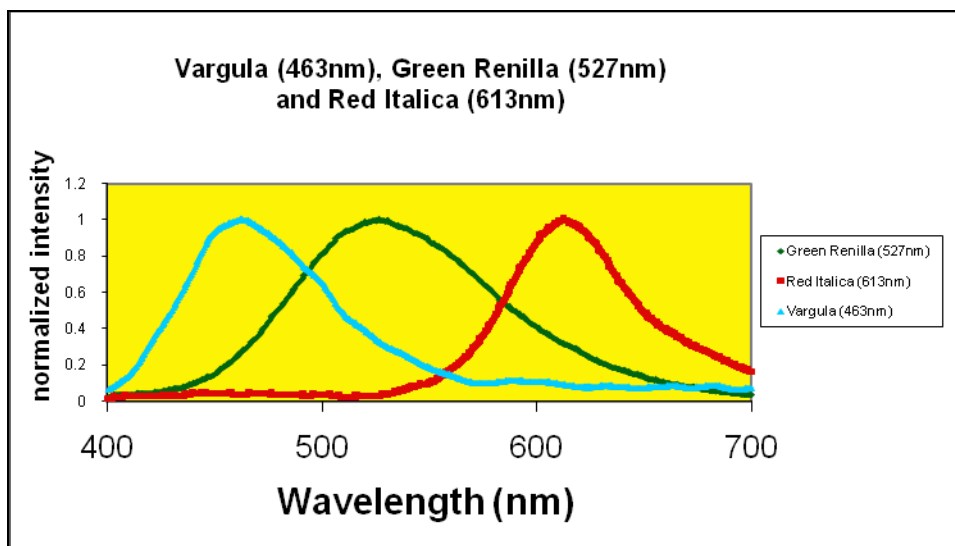


Figure 5: Emission spectra of different luciferases in samples of transfected cell lysates. Relative luciferase activities of Cypridina, Renilla and Red Luciola luciferases were assayed with the appropriate luciferase assay reagent to obtain spectral profiles. The emission max of Vargula luciferase is 463 nm; Green Renilla luciferase is 527 nm and Red Luciola luciferase is 617 nm. (Data courtesy of Justin Rosenberg, Dr Bruce Branchini's lab, Connecticut College, USA)

Ordering information:

Please visit our web link for a complete listing of Luciola firefly luciferase-based expression vectors, assay reagents and related products

<http://targetingsystems.net/pricing.html>

For more info call technical support at 1-866-620-4018

Or customer service 1-619-562-1518 or use our website www.targetingsystems.net