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Our newest offering

LiveResponse Luciferase Assay System

The smart solution for ex-vivo monitoring of in vivo processes .
Now you can screen the same compounds for effects on multiple pathways in real time using the same group of transfected cells and still keep cells alive for downstream applications.

The LiveResponse advantage:
A great selection of secreted luciferase reporters, a great set of assay reagents, with a choice of several assay formats.

A panel of 5 novel secreted luciferases- The brightest and the best. Study multiple pathways in living cells or animals by analyzing activities of up to 5 different promoters, each controlling expression of a different secretable reporter. Also provided are assay reagents for all 5 luciferases

The LiveResponse Reporters:

The largest selection of secretad luciferase reporters:

- Gaussia Princeps Luciferase
- Red-emitting Firefly luciferase (Luciola Italica)
- Cypridina (Vargula) luciferase
- Green-emitting Renilla Luciferase Blue-emitting Renilla luciferase

A set of five promoter-less vectors expressing secreted luciferases is provided in each kit The luciferase reporters include Gaussia Princeps luciferase, Cypridina (Vargula) luciferase, a novel secreted red-emitting luciferase and two novel color-shifted mutants of Renilla luciferase with improved properties. A different promoter can be sub-cloned into each luciferase expression vector thereby allowing analysis of multiple gene expression patterns within the same cell in response to the same set of inducers or suppressors of gene function. A brief description of the different Luciferase reporters is given below:

A novel red-emtting secreted luciferase:

Central to the versatility of the LiveResponse system is our newest reporter- a genetically engineered modification of the red Luciola Italica luciferase which is about1000 times brighter than the native luciferase and expressed efficiently both as an intracellular and secretable reporter. A secretable reporter was developed by introducing a secretory tag at the amino terminal end of the red luciferase. The robustness and stability of the bioluminescent signal allows for excellent multiplexing with blue and green emitting secreted luciferases. The unique properties of this secreted red luciferase in terms of sensitivity, stability and luminescent intensity suggest it to be very attractive for in vivo imaging applications on account of it's intense intracellular signal. Imaging of deep seated tissue such as brain tumors and tracking implanted stem cells are two potential applications.

Two secreted color-shifted (blue-shifted and green-emitting) secreted mutants of Renilla luciferase

Renilla luciferases cloned from the soft coral sea pansy Renilla Reniformis catalyze a bioluminescent reaction that utilizes coelentrazine as a substrate. Unlike firefly luciferases, the coelenterazine-utilizing luciferases do not require accessory high energy molecules such as ATP for their signal, simplifying their use in a number of reporter applications.

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Mutants of Renilla luciferase with improved properties have been developed for expression as secreted reporters with the goal of establishing multiplexed luciferase assays 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 blue-shifted variant although less brighter than the green variant, has an emission max of 467 nm and can be conveniently multiplexed with the red Italica 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.

In contrast the widely used Renilla-firefly dual reporter system is more expensive and takes longer handling time as two different solutions are used to assay the two different reporters. Further it is necessary to lyse cells prior to the assay . In addition to increasing assay time, this assay format requires that a separate group of transfected cells be lysed to measure gene expression at each time point.

Cypridina (Vargula) Luciferase

Cypridina luciferase, a secreted luciferase form the ostracod Cypridina (Vargula Hilgendorfi) although cloned in 1989 is now commercially available for the first time as a secreted reporter for studying gene expression in mammalian cells. Vargula luciferase with an emission max of 463 nm is as bright as Gaussia luciferase and an excellent complement in multiplexesd assays with a red-emitting luciferase or with Gaussia luciferase. Cypridina luciferase utilizes a different substrate Vargulin (Cypridina luciferin) which makes it suitable for multiplexing with other luciferase components of the LiveResponse system that utilize coelenterazine or firefly luciferin as a substrate.

Gaussia Princeps Luciferase :

Gaussia luciferase, from the marine coepopod Gaussia Princeps, a coelentrerazine utilizing luciferase was the first mammalian luciferase expression system developed and commercialized by Targeting Systems. The Gaussia gene has been licensed form Prolume Inc, AZ. The versatility of Gaussia luciferase and it's usefulness as a secreted reporter has been documented in several publications (see references) . Although Gaussia luciferase is over a 1000-times brighter than the photinus luciferases, the Vargula luciferase are close in brightness to Gaussia luciferase in the stable bioluminescence format of the assay. The red-emitting secreted Luicola firefly luciferase is not as bright as Caussia luciferase Cyptidina luciferase systems but still offers a very robust stable luminescent signal

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Assay Reagents:

The LiveResponse kit comes with 3 assay reagents that enable one to conveniently measure all the 5 LiveResponse luciferase reporters.

Gaussia/Renilla luciferase assay reagent GRLAR : An assay reagent suitable for assaying both Gaussia and Renilla luciferase in cell supernatants

Firefly luciferase assay reagent (FLAR-1): An assay reagent for assaying Firefly luciferase. A homogenous one step assay format suitable for high throughput applications (30% decay in 2 hours). Also suitable for dual assay of both Luciola luciferases Pl,ease download the firefly luciferase product brochure from our website for more info.

Vargula (Cypridina) luciferase assay reagent (VLAR-1): An assay reagent for Vargula luciferase- based assays, Download the Vargula luciferase product brochure for more info.

Assay Formats:

A choice of several assay formats.

Now you can measure up to 4 secreted luciferases using separate assay reagents based on differences in substrate specificities of the three luciferase reporters.,

A better choice: Measure two or three compatible LiveResponse luciferase reporters using a single assay solution. If desired, two or three of the assay reagents described above can be mixed together in an appropriate combination to simultaneously measure two or three different luciferase reporters in the same sample and spectrally resolve the different luciferase activities (based on differences in emission maxima) using appropriate filters.

Figure 1: Kinetics of luciferase activity of different luciferase reporters using luciferase assay reagents in the LiveResponse panel

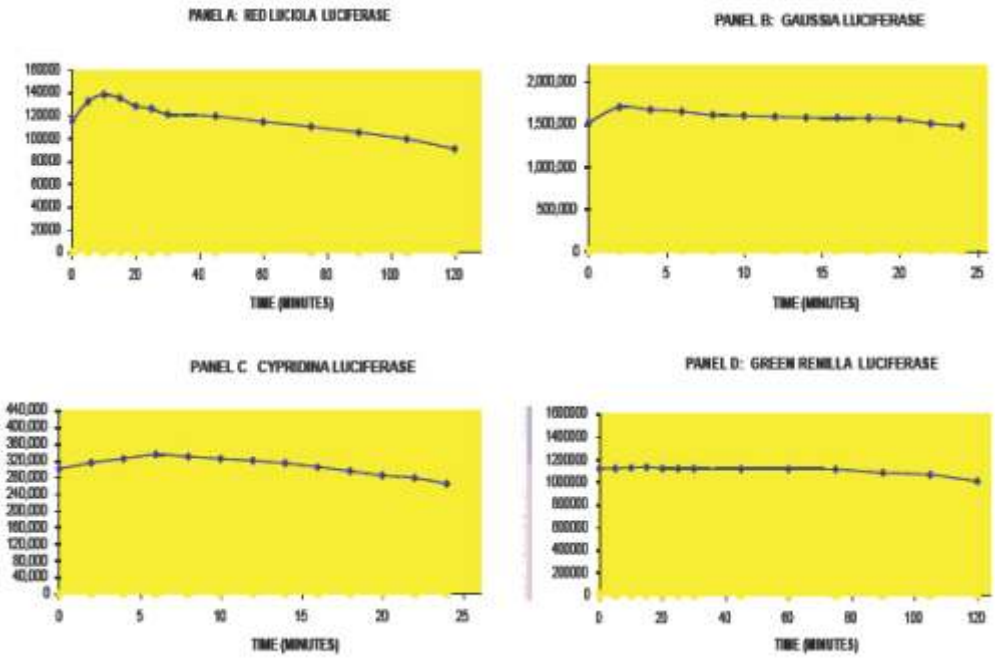

Figure 2: Reactions were set up to measure the kinetics of the luciferase activities of different luciferases in samples of transfected cells. Luciferase activities were assayed using the luciferase assay reagents supplied with the LiveResponse assay kit. Panel A: Red Luciola (firefly) luciferase, Panel B : Gaussia Princeps luciferase, Panel C: Cypridina luciferase and Panel D: Green Renilla luciferase. Data represents mean of triplicate determinations.

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Multiplexed Assay Formats available within the LiveResponse System

Several sual luciferase reporter options are offered in the LiveResponse systems. The two luciferase activites can be spectrally resolved using a single assay solution and appropriate filters or measured separately using different subtrates.

Figure 3A : Dual Luciferase assays based on spectral resolution of luciferase activities

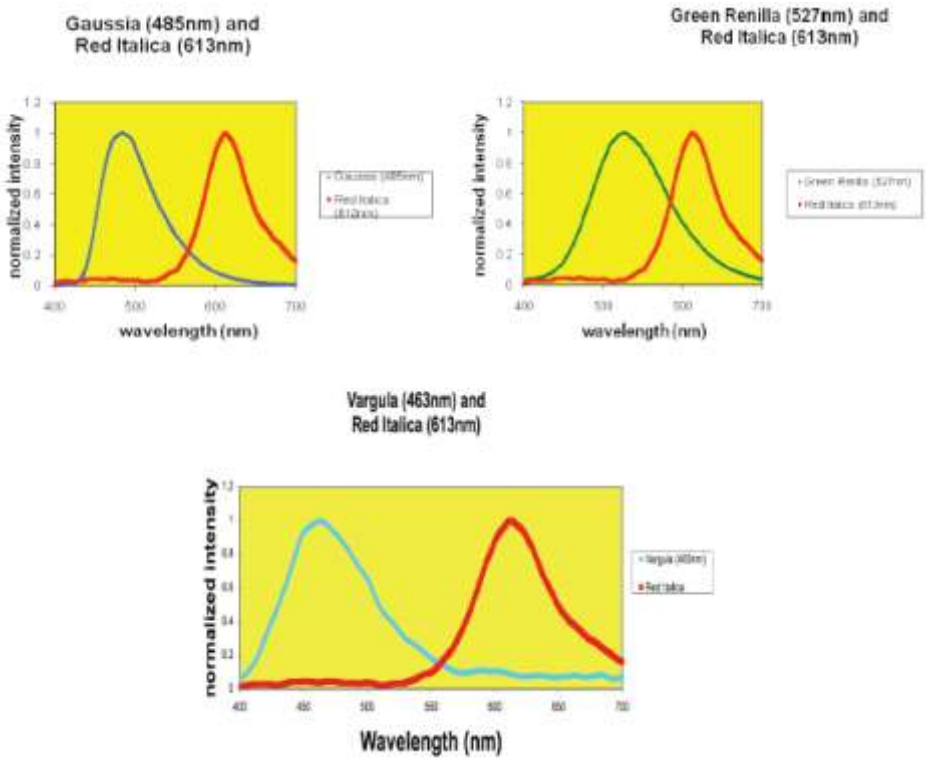

Figure 3A: Emission spectra of different luciferases in samples of transfected cells (lysates or supernatants). The emission spectra were recorded on a Fluorolog-3 spectrofluorometer (Horiba Scientific, Japan) using a liquid nitrogen cooled CCD. The luciferases were assayed by mixing 200 ul of the sample with the appropriate luciferase assay reagent to obtain spectral profiles. Emission max of Gaussia luciferase is 482 nm; Green Renilla is 527 nm; Cypridina Luciferase is 463 nm; Red italica 617 nm

Dual luciferase reporter assay based on gaussia luciferase and Red Luciola Italica luciferse:

This is the dual assay system of choice as it provides the greatest sensitivity by using a combination of two of the brightest luciferases in combination with optimal peak separation and stable bioluminescent signals. Note that this luciferase combination allows for the versatility of either an assay format wherein the two luciferases are assayed separately or using a single assay solution prepared by mixing the Gaussia assay reagent with the firefly luciferase assay reagent into a single solution.

Dual luciferase reporter assay based on Cypridina luciferase and Red firefly luciferas:

This luciferase combination allows for the versatility of either an assay format wherein the two luciferases are assayed separately or using a single assay solution prepared by mixing the Gaussia assay reagent with the firefly luciferase assay reagent into a single solution.

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Dual lucifersae reporter assay based on Green Renilla luciferase and Red filrefly lucierase:

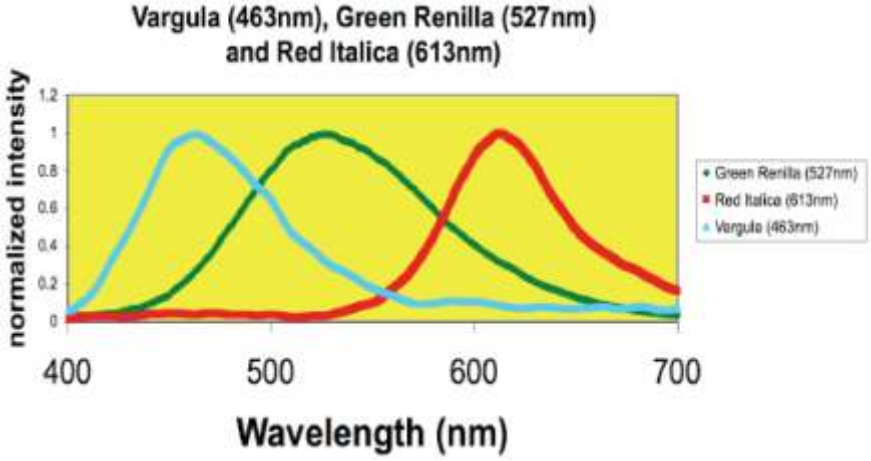
This luciferase combination allows for the versatility of either an assay format wherein the two luciferases are assayed separately or using a single assay solution prepared by mixing the Gaussia assay reagent with the firefly luciferase assay reagent into a single solution. Howevere since there is significant overlap of Green renilla luminescent signa and the Red firefly luciferase in the 550 to 605 nm range the filters need to be careflully selected. However the assay can be conveniently performed by measuring the two luciferases separately in the supernatant media of transfected cells.

Dual luciferase reporter assay based on Gaussia luciferae and Cypridina luciferase:

This is an excellent reporter combination of two luciferases which use different substrates and are very bright. And efficiently secreted . However, one limitation of this assay is that the emission max of Cypridina luciferase (463 nk) is very close to that of Gaussia luciferase (482 nm) making it difficult to spectrally resolve the two luciferase acitivties. The assay can therefore only be carried out in a single assay or sequential format.

Triple Luciferase Reporter Assay

Vargula luciferase, Green Renilla luciferase, Red Italica luciferase



Vargula luciferase -
Emission max 463 nm

Green renilla Luciferase -
Emission max 527 nm

Red firefly luciferase -
Emission max 613 nm

Figure 3B: 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

This assay system is the most attractive triple luciferase reporter assay system as it offers a combination of very bright luminescent signals as well as flexibility in assay formats. Since the three luciferase reporters utilize different substrates, they can be assayed using separate reagents or the three assay reagents can be combined into a single assay solution that measures activities of all three luciferases and then distinguishes them by spectral resolution. Due to differences in the stability of the luminescent signals Vargula luciferase must be measured first followed by Renilla and firefly lcuiferase. It is preferable to have the expression of the green Renilla or red firefly luciferase controlled by the strongest promoters.

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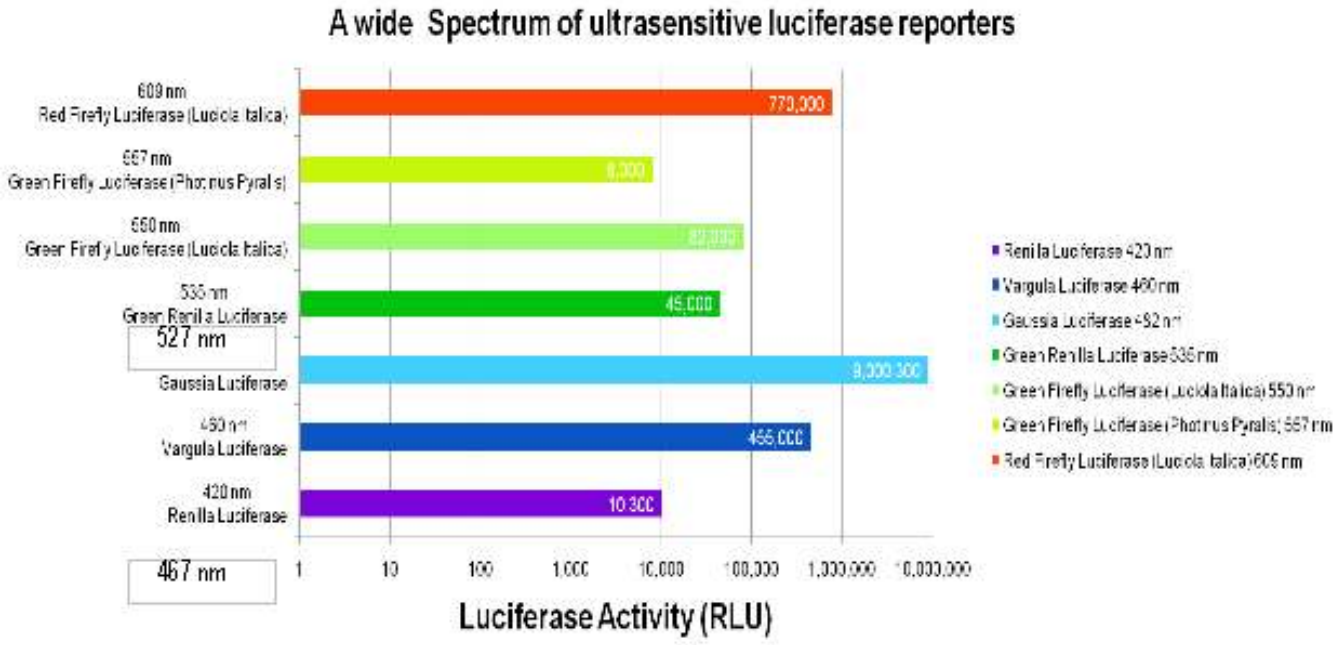
Triple luciferase reporter assay based on differences in subtrate specificities (Gaussia luciferase, Cypridina luciferase and Red Luciola Italica luciferse):

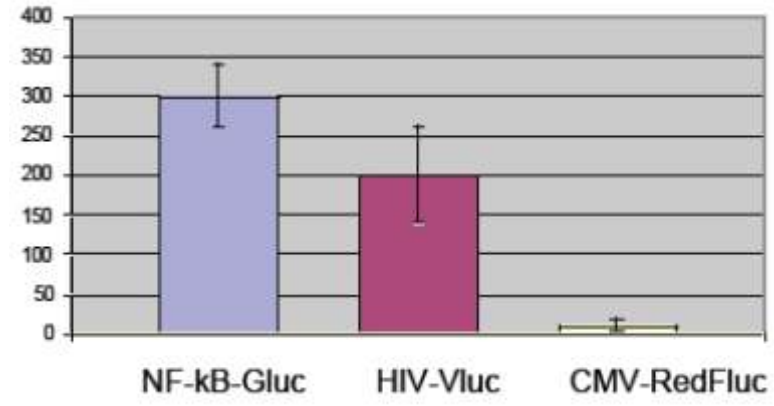
Emission maxima and relative luciferase activity profiles of the secreted luciferase panel components fo the Liveresponse System



A comparision of the Luciola Italica red and green-emitting luciferasese with other luciferase reporters.: The experiment was conducted in HEK-293 cells.using either Gaussia luciferase or Vargula luciferase as the denominator plasmid. **Of the above 5 reporters (Red-emitting Italica lucifersae, Blue and green renilla luciferases, Gaussia and Vargula luciferase are available as secreted reporters.**

Figure 5: Induction of HIV promoter-driven Cyrpridina luciferase expression by the TAT protein (Panel A) and induction of NF-kB –driven Gaussia luciferase expression by TNF-alpha (Panel B) in HEK-293 cells

Panel A: Induction of HIV promoter by the TAT protein



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Panel B: Induction of NF-kB signalling by TNF-alpha

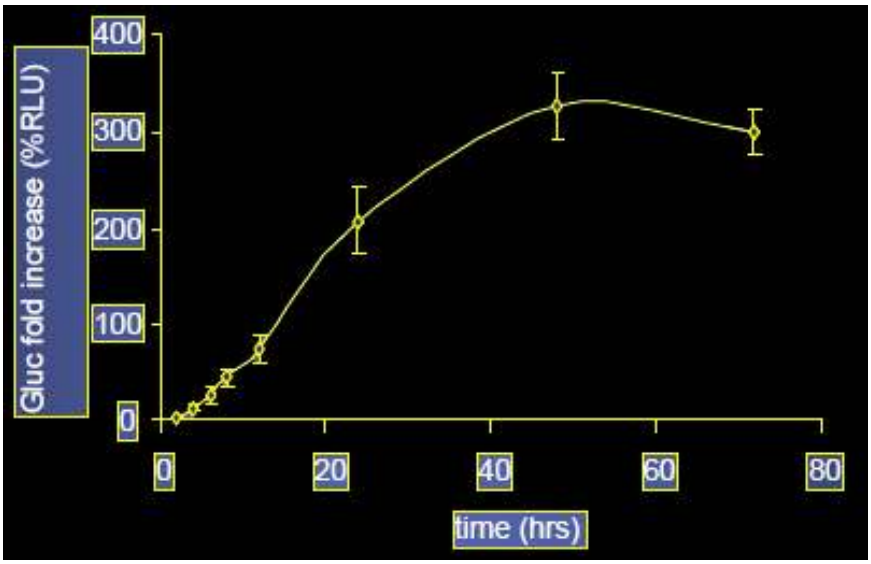

Figure 5: HEK 293 were transduced with 3 vectors carrying expression cassette for: (1) Gaussia luciferase under control of NF-kB responsive promoter (NF-kB-Gluc); (2) Cypridina-luciferase under control of HIV1-promoter (HIV-Vluc); (3) red-emitting firefly luciferase under control of CMV promoter (CMV-RedFluc). These cells were then transfected with the SV40-pTAT plasmid (which activates HIV promoter) and treated with 15 ng/ml TNFa (which activates NFkB). 24 hrs post-treatment, aliquots of conditioned medium were assayed for Gaussia and Cypridina luciferase activity. Cell lysates were also assayed for RedFluc activity which was used as a viability marker. Signals were normalized to RedFluc activity and plotted as % fold increase in which the untreated samples was set to 1%. (B) real-time monitoring of NFkB activation using Gaussia luciferase. Cells expressing NFkB-Gluc were treated with 2.5 ng/ml of TNFa. Aliquots of conditioned medium were assayed for Gluc activity at different time points.

Components and pricing:

LiveResponse Luciferasae Assay System

Catalog # LVR-001, Introductory price $2000

A panel of 5 promoterless vectors with a multiple cloning site for subcloning the promoter of interest upstream of the luciferase gene:

pBasic SRedFluc (20 ug) ------ Red firefly luciferase expression vector
pBasic-Gluc (20 ug) ---------- Gaussia lcuiferase expression vector
pBasic—Vluc (20 ug) ---------- Vargula luciferase expression vector
pBasicBrenLuc (20 ug) --------- Blue-shifted Renilla luciferase expression vector
pBasicGrRenLuc (20 ug) ------- Green-emtting Renilla luciferase expression vector

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Assay Reagents:

Gaussia/Renilla Luciferase Assay Reagent (1000 reactions)
Vargula (Cypridina) Luciferase Assay Reagent (1000 reactions)
Firefly Luciferase Assay Reagent (1000 reactions)

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

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 tour website www.targetingsystems.net

For a list of citation describing the use of these luciferase reporters please access the following link on our website:

http://targetingsystems.net/drug-discovery.html

Note: This product is being sold for research purposes only. For commercial use of this product please contact technology transfer

At 1-866 620 4018. This product line is covered by several issued and pending patents

Commercial use of the Gaussia luciferase gene requires a license from Prolume Inc, Arizona.
Please contact Li Bryan at 928 367 1200