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An assay system for Vargula (Cypridina) luciferase

Another secreted luciferase – a perfect compliment to Gaussia luciferase fpr a dual luciferase assay

Applications:

? Single and multiplexed Luciferase assay systems for high throughput screening.

? Mammalian expression vectors for studying regulation of gene expression

? 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 form 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.. The dual renilla luciferase assay reagent is a single solution-based assay system that uses two improved Renilla luciferase mutants

Principle of the Luciferase Assay

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.

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 blue

shifted variant has an emission max of 467 nm and can be conveniently multiplexed with the green variant. 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.

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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 ot increasing assay time, this assay format requires that a separate group of transfected cells be lysed to measure gene expression at each time point.

The use of secreted luciferases previously available has been limited. This However, has changed with the introduction of Targeting Systems' "LiveResponse" Luciferase reporter panle 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 reporte)r 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.

Specifications of the Dual renilla luciferase assay:

Linear range: Assay linear over eight orders of magnitude

Limit of detection: less than 1 fg of luciferase per sample

Product profile: Targeting Systems offers a one-step luciferase assay kit to facilitate measurement of luciferase activity in HTS (high throughput applications). To increase shelf life of the reagent the substrate coelenterazine is provided as a 100 X solution that should be diluted with the assay dilution buffer just before use.

Storage Temperature: We recommend storage at -20 0 C. The assay dilution and lysis buffers may be stored at 4 o C if desired

Stability: We guarantee stability for 1 year from date of purchase

Advantages:

- Assaying both reporters with a single solution increases speed and minimizes handling
- 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
- 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.
- Can be conveniently multiplexed with other secreted luciferase reporters (see brochure on LiveResponse system on our website)

Protocol:

ASSAY CELL SUPERNATANTS IN 96-WELL FORMAT: Transfer 5-20 ul aliquots of the supernatant media from transfected cells, mix with 50 ul of the DRLAR reagent and assay. For an end point assay add 50 µl of the DRLAR-1 reagent to 50µl of the supernatant culture media from cells grown in multi-well tissue culture dishes, mix and read in a lumionometer or microplate reader. Read activity of the blue-emitting renilla luciferase first.

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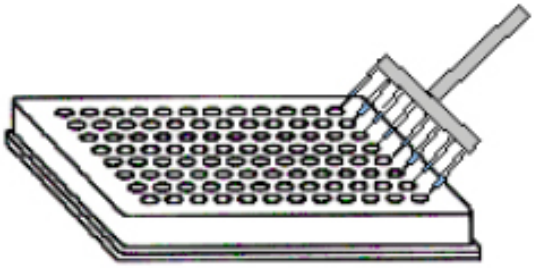
ASSAY OF LUCIFERASE ACTIVITY IN CELL LYSATES: The DRLAR luciferase assay reagent can also be used to measure luciferase activity in pre-lysed cells. NOTE: If you need to measure intracellular luciferase activity lyse cells first use the cell lysis buffer from Targeting Systems . Dilute the 5X CLR buffer 1:5 with water. Add enough lysis buffer to cover cell. Aspirate cell cultre media and wash cells twice with serum free DMEM. Add enough of 1X lcell lysis buffer to cover cells. Shake for 20 min at 400 rpm on an orbital shaker (room temperature). 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. All assay reagents should be close to room temperature at the time of assay.

Simple One-step Assay

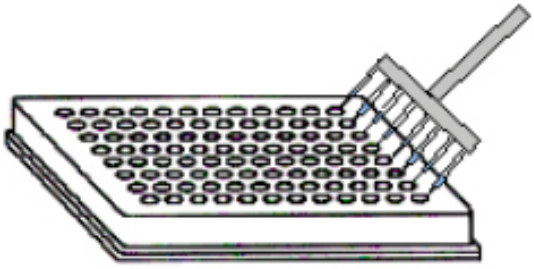


Aspirate cell
culture media and transfer
to a new set of wells

DRLAR

Add dual renilla luciferase assay reagent.
Mix and read samples in the luminometer.
Using appropriate filters for spectral resolution

Optional Assay Format



DRLAR

Add dual Renilla luciferse assay reagent directly to cell supernatants (50 µl per well) , mix and read in luminometer using appropriate filters for spectral resolution

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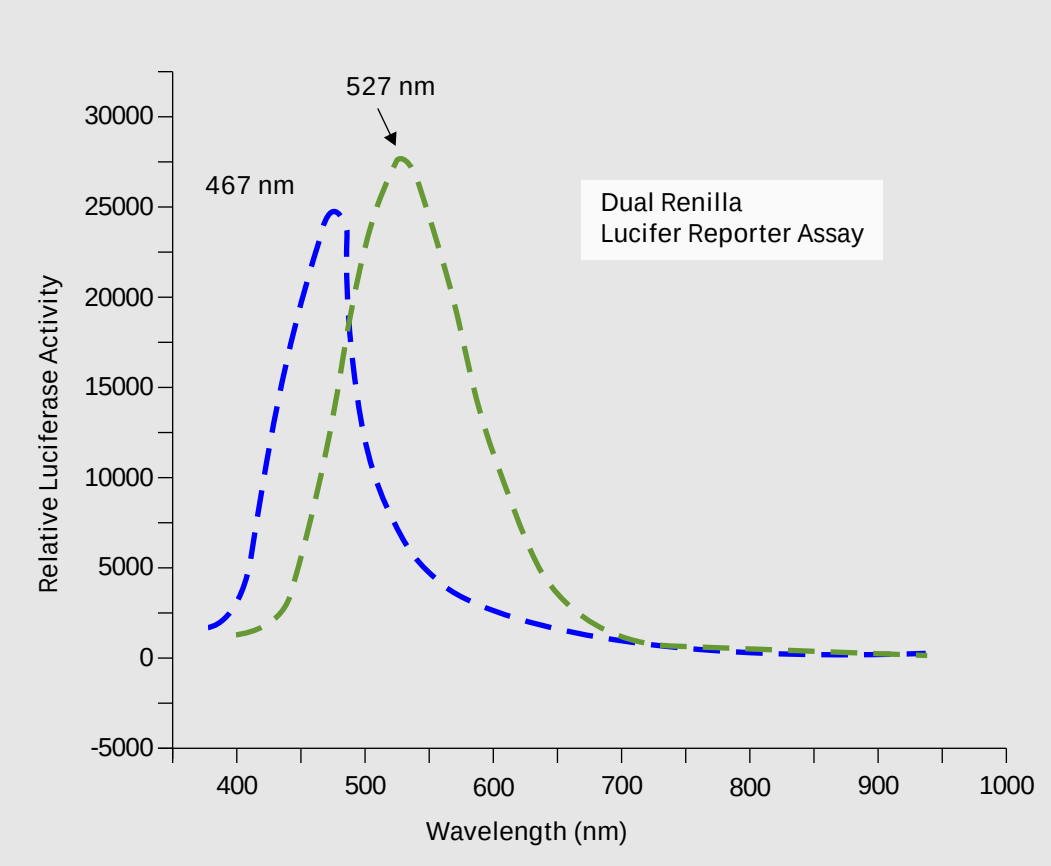
Emission spectra of Blue- and Green-emitting Renilla luciferases



Blue renilla – Emission max 467 nm

Green renilla Luciferase – Emission max 527 nm

Important Considerations For Optimal Resolution

Please note that the activity of the Blue emitting Renilla luciferase as shown in the figure above is approx 50% of the green emitter. However it is easily possible to adjust the relative intensity of the signals to achieve better reolution of the peaks . In this case the recommendation would be to use the green –emitting Renilla luciferase under control of a weaker promoter and the Blue–emitting Renilla luciferase under control of a strong promoter. Alternately one can just use smaller amounts of the expression vector expressing the green emitting Renilla luciferase to obtain better resolution.

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Dual Renilla Luciferase Assay System DRLAR-1

Product	Cat. #	Size	Unit Price
Luciferase assay kit	DRLAR-1	100ml	$420
DRLAR-1	DRLAR-1L	100ml	$3500

Special discounts available for bulk purchasesProduct

DESCRIPTION: The du Renilla luciferase assay kit DRLAR-1 is a homogenous luciferase gene detection system. At different times after transfectiion , aliquots of the supernatants can be withdrawn, transferred to a new set of wells, mixed with 50 ul fo the DRLAR reagents and read in the luminometer using appropriate fitlers to spectrally resolve the blue emitting (Emission max 475 nm) and green emitting (emission max 527 nm) Renilla luciferases

The DRLAR-1 luciferase assay kit can also be added directly to cell supernatants of transfected cells for an end point assay.

The DRLAR reagent can also be used to measure luciferase activity in pre-lysed cell extracts using our lyciss buffer (see attached protocol sheet). The assay is compatible with both sample processing robots and with reagent injectors in many luminometers

Specifications:

- Reproducibility – CV less than 5%
- Linear range- Assay linear over seven orders of magnitude
- Limit of detection – less than 1 fg of luciferase per sample
- No disposal problems or hazards are associated with the use of these luciferase assay kits

Storage: Store reagents at –20° C or –70° C

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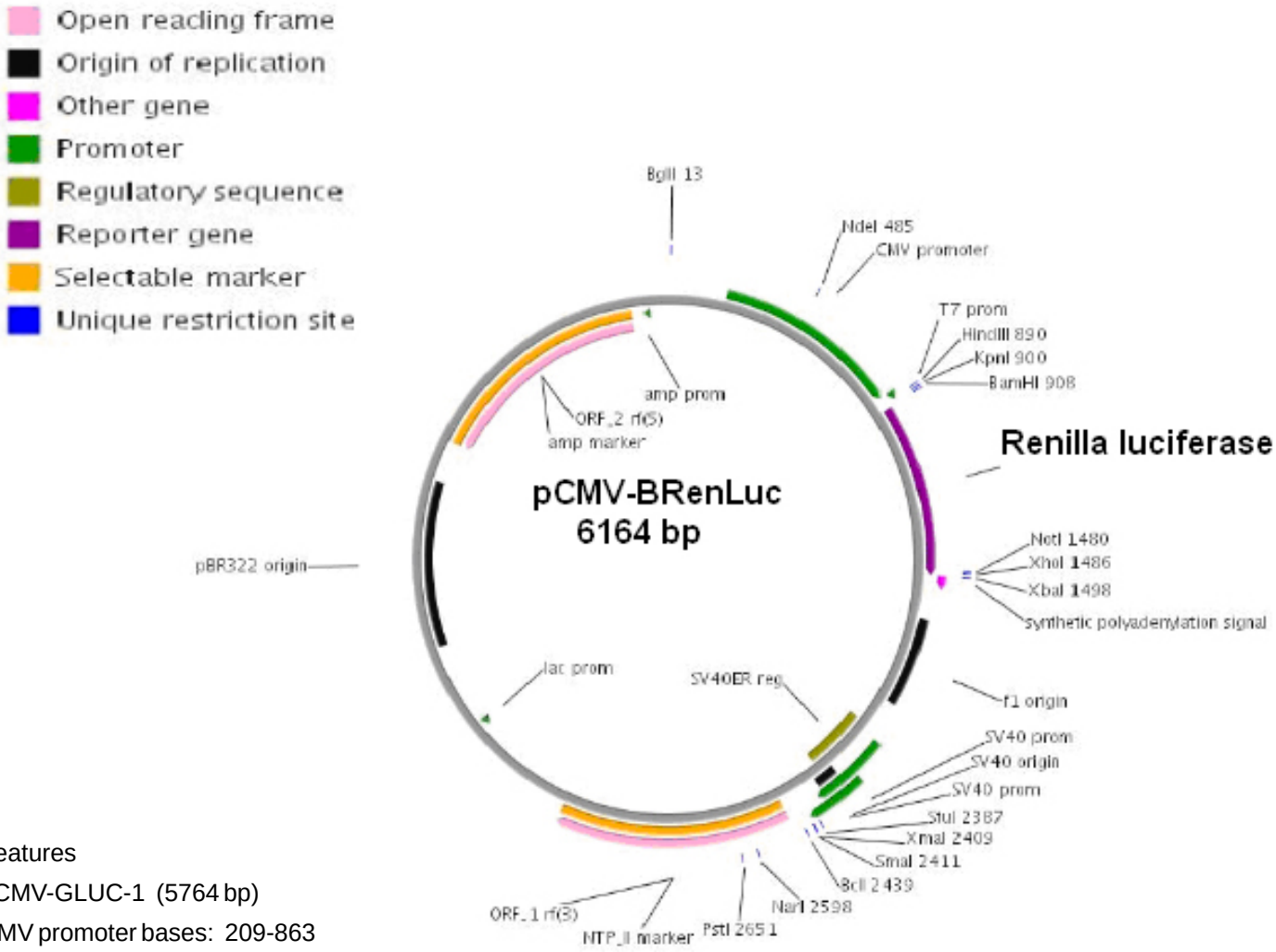
Expression vectors expressing the Blue-shifted and Green Renilla luciferases mutants

pCMV-BRenluc- Expression vector expressing Blue-emitting luciferase under control of CMV promoter



Features

pCMV-GLUC-1 (5764 bp)

CMV promoter bases: 209-863

Blue-emitting Renilla luciferase gene: 907-1897

T7 promoter bases: 864-882

Polylinker bases: 889-907

SP6 promoter: 1913-1930

Synthetic polyadenylation site: 1897-1941

SV40 promoter bases: 2482-2817

SV40 origin of replication: bases 2596-2681

Neomycin ORF : bases 2853-3647

SV40 PolyA: bases 3702-4074

ColE1 origin: bases 4334-5007

Ampicillin ORF: bases 5152-6012

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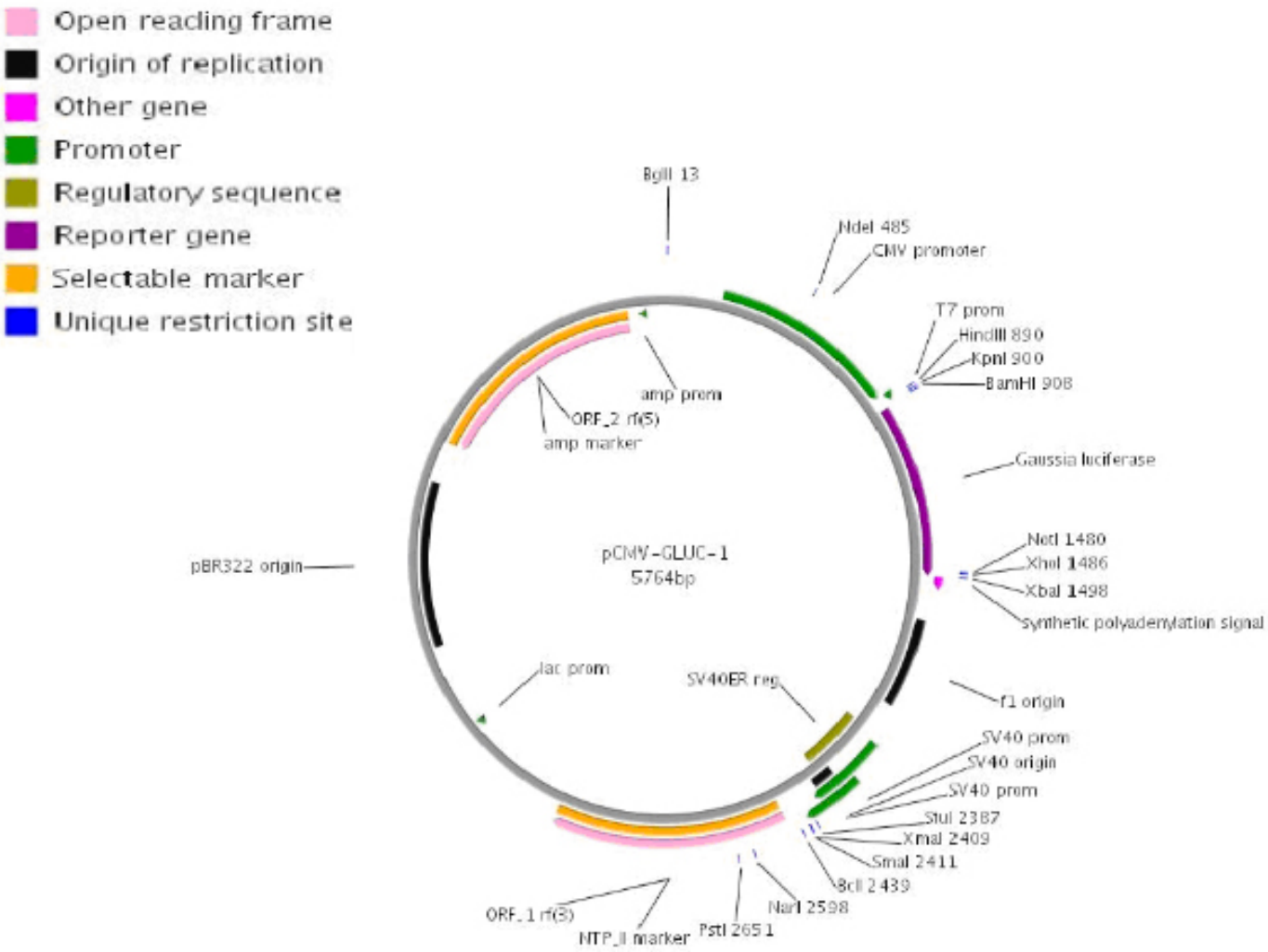
PCMV-GrRenluc- Expression vector expressing Green-emitting luciferase under control of



Green-emitting Renilla luciferase gene: 907-1897
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Synthetic polyadenylation site: 1897-1941
SV40 promoter bases: 2482-2817
SV40 origin of replication: bases 2596-2681
Neomycin ORF : bases 2853-3647
SV40 PolyA: bases 3702-4074
ColE1 origin: bases 4334-5007
Ampicillin ORF: bases 5152-6012

The plasmid map shown above was constructed with the help of the following link:
http://wishart.biology.ualberta.ca/PlasMapper/jsp/displayPlasmidMap.jsp?fileName=plasMap115_1101977745436.jpg&fileFormat=jpg
Xiaoli Dong, Paul Stothard, Ian J. Forsythe, and David S. Wishart "PlasMapper: a web server for drawing and auto-annotating plasmid maps" Nucleic Acids Res. 2004 Jul 1;32(Web Server issue):W660-4.

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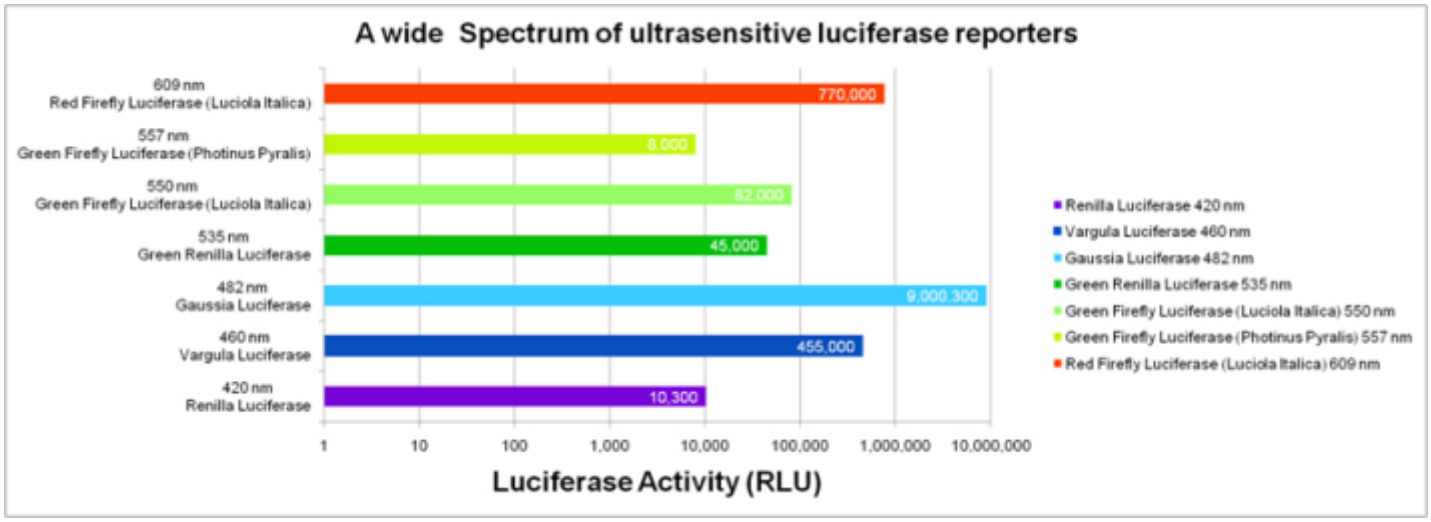
The CMV promoter is covered by U.S. Patents 5,168,062 and 5,385,839 and its use is permitted for research purposes only. Any other use of the CMV promoter requires a license from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.

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 patnets. Commercial use of these products requires licenses from Targetimg Systems. And other sources.

Related Products



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.

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 upto 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.

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For more information please visit our website and click on the revolving LiveResponse banner on the home page.

Other Multiplexed assays:

The emission spectra and multiplexing options available with some of our novel luciferase reporters are given below:. Central to all our mulitiplexed assays is a very bright secreted red=emitting luciferase (almost as bright as Gaussia luciferase whose emission max at 613 nm makes it ideal for multiplexing with the more common blue and green emitting luciferases when compared to the luminescent activity of Gaussia luciferase assayed using the stabilizing versions of the assay reagent.

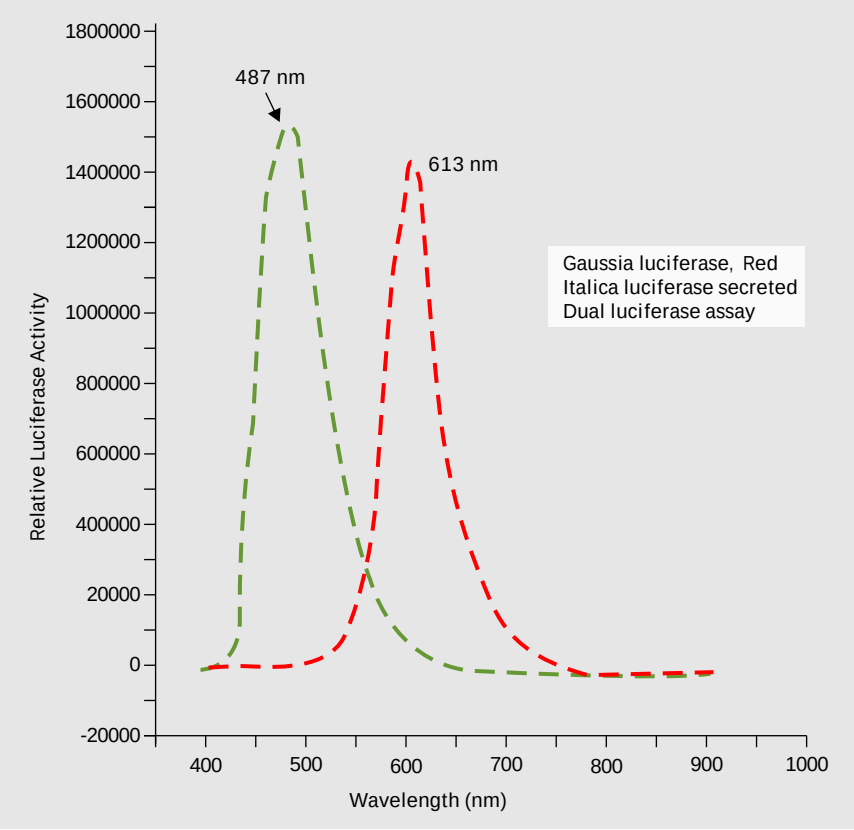
Another factor that makes our multiplexed assays different is that multiple luciferases can be assayed in the same sample with a single assay solution based on spectral resolution of 3 or 4 different luciferase acitivties. This simple assay format significantly reduces handling time and increases speed of assay

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 componenets of the LiveResponse system to assass state of transfected cells by simply subcloning the NFKappa B or CREB upstream of the luciferase gene in the pCMV-Red Flcu vector

Dual Luciferase Reporter Assay

Gaussia luciferase, Red Italica luciferase



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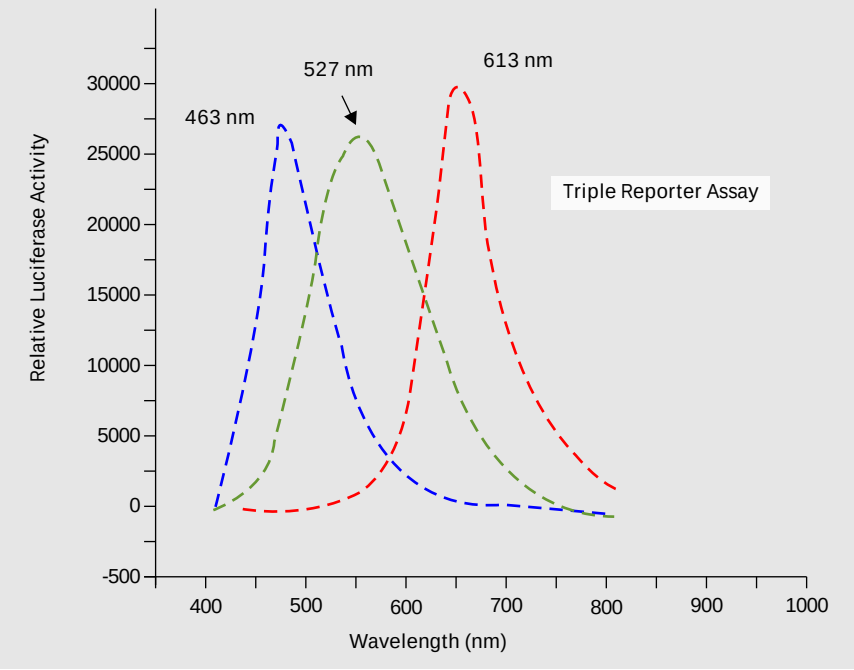
Gaussia Princeps Luciferase – Emission max 482 nm
Red firefly luciferase- Emission max 613 nm

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 Vargula luciferase can easily replace Gaussia luciferase or be considered as a thirs reporter using either a separate assay solution or a stop and glow 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

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 wesite www.targetingsystems.net