

Expression vectors and assay systems for Red and Green-emitting luciferases from the Italian firefly *Luciola Italica*

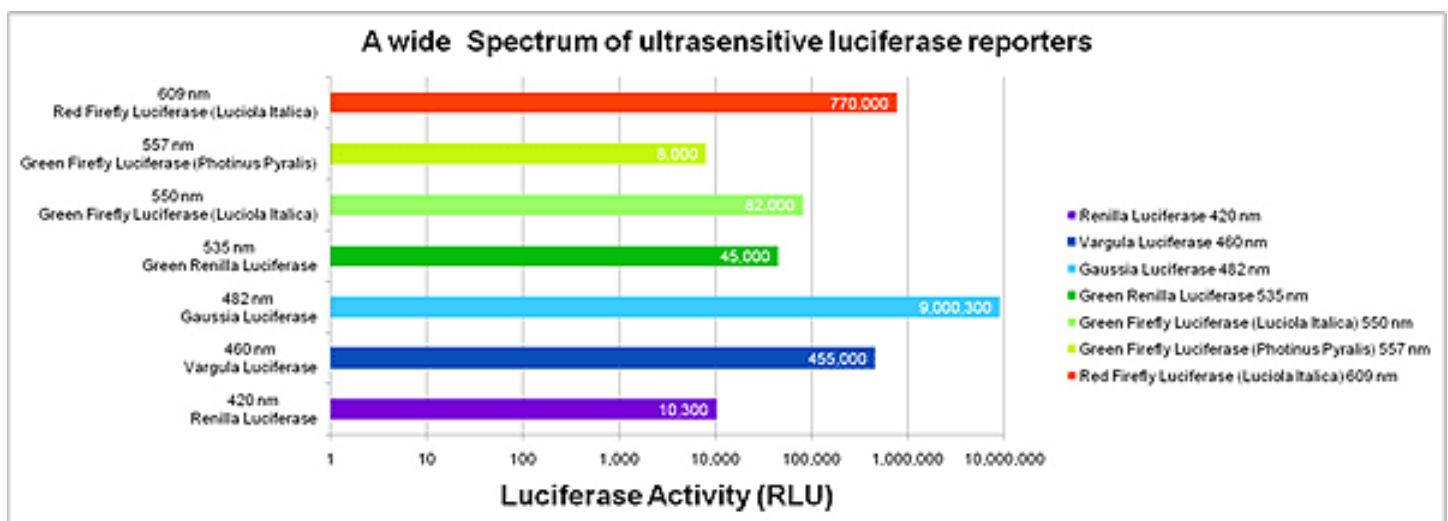
Improved properties: Better stability, multiplexing options and brighter signals

Applications:

- ◆ Single and multiplexed luciferase assay systems for high throughput screening.
- ◆ Mammalian expression vectors for studying regulation of gene expression
- ◆ The red-emitting *Luciola* luciferase (emission max 617 nm) is an excellent reporter for in vivo imaging applications and analysis of gene expression in vivo.
- ◆ Some of the multiplexed assay systems based on the red-emitting *Luciola* 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).

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.



FLAR-1 Luciferase Assay Reagent- Quick Protocol

The FLAR-1 Reagent provides at least more light output than other extended half-life luciferase reagents, depending on the cell line and medium used. Signal half-life is approximately 30 minutes in commonly used cell culture media, which is sufficient to read a 96-well plate with less than 5% signal decay.

1. Remove 96- or 384-well plates containing mammalian cells from the incubator. The plates used must be compatible with the luminometer being used. For best results, equilibrate cultured cells to room temperature before performing Step 2.
2. To each well add a volume of FLAR-1 Reagent equal to the volume of culture medium in the well, and mix. (For 96-well plates, typically 100µl of reagent is added to cells in 100µl of culture medium. For 384-well plates, typically 30µl of reagent is added to cells grown in 30ul medium)
3. Wait at least 2 minutes to allow cell lysis, then measure luminescence in a luminometer

Characteristics of the FLAR-1 luciferase Assay Reagent.

| | |
|------------------|---|
| Format | Homogenous or non-homogenous |
| Process | Continuous |
| Sensitivity | Maximum light output |
| Signal Half-Life | 30 minutes (Photinus Pyralis luciferase) to 1 hour (Luciola Italica Luciferase) |
| Cell lysis time | 2 mins |

NH = Nonhomogeneous assay format (when used with 5X Cell Lysis Buffer, (Cat.# CLR-01).

H = Homogeneous assay format.

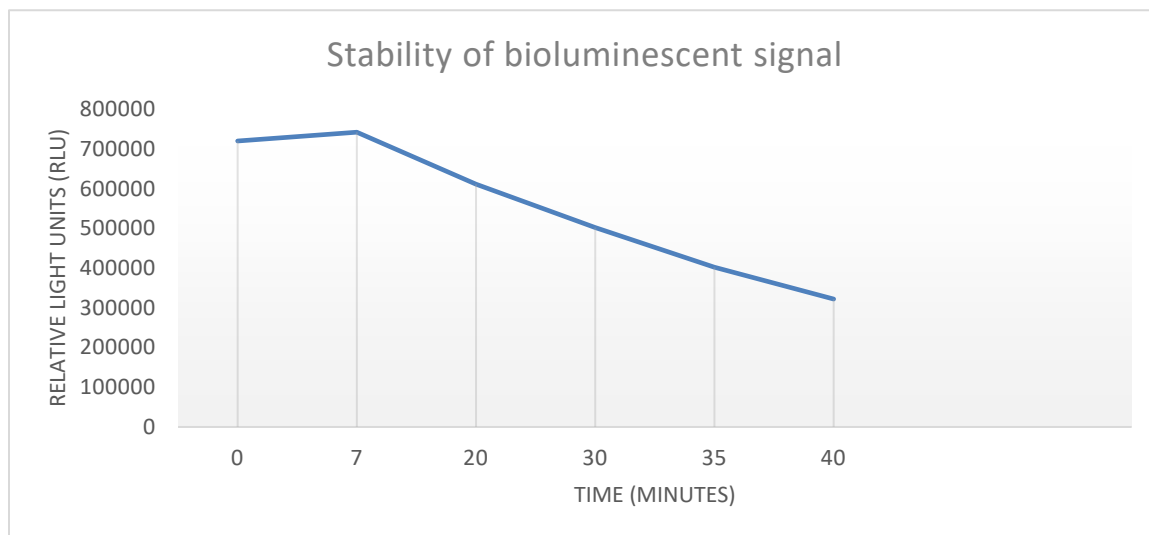


Figure 1: Stability of the bioluminescent signal using the FLAR luciferase assay reagent). Results are expressed as the mean of quadruplicate determinations (CV less than 5%). The intensity of the bioluminescent signal using the FLAR reagent from Targeting Systems is comparable to the intensity of the bioluminescent signal in assays using Promega's Bright glo reagent. In the experiment shown above the FLAR reagent was directly added to the supernatant media.

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 5-20 µl of luciferase containing sample or cell lysate with 100 µl of the luciferase assay kit (FLAR-1) and read immediately in the luminometer. All assay reagents should be close to room temperature at the time of assay.

NOTE: If you need to lyse cells first use the 5X CLR cell lysis buffer from Targeting Systems, (catalog no 5X CLR-01)

Protocol for cell lysis and luciferase assay:

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 (50ul for 96-well, 300ul for a 12-well, 800ul for a 6-well dish and 3 ml for a 10cm 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.
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 targsys@aol.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.

Firefly Luciferase Assay reagent (FLAR-1)

Brightest Signal Intensity, best signal stability - For HTS applications

Firefly luciferase expression vectors and assay reagents for drug discovery applicaitons

| Catalog no. | Size | Description | Price |
|-------------|-------------|--|----------|
| FL-001 | 20 ug | pCMV-RedFluc, expresses red-emitting firefly luciferase under control of the CMV promoter | \$299 |
| GFL-001 | 20 ug | pCMV-GrFluc, expresses green-emitting firefly luciferase under control of the CMV promoter | \$299 |
| FLAR-1 | 1000 assays | Firefly luciferase assay reagentBright stable version | \$400.00 |
| DLAR-2B | 1000 assays | Firefly-Renilla luciferase dual assay reagent | \$400.00 |

Please call for special pricing on bulk reagent purchase.

Related Products: For a list of firefly luciferase based lentiviral products and ready-to-use lentiviruses click on the following link on our website <https://www.targetingsystems.net/cell-tracking.php>
<https://www.targetingsystems.net/product-price-list.php>

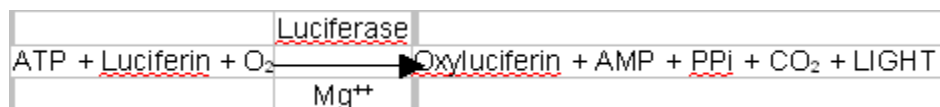
Product Description:

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 firefly luciferase activity in both supernatants or lysates 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 luciferin substrate and the bioluminescence stabilizers all included in a single assay reagent.

Specifications:

Linear range- Assay linear over seven orders of magnitude

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.

Multiplexing Options: Several options for dual and triple luciferase assays based on combinations of firefly luciferase with Renilla luciferase, Gaussia luciferase or Cypridina luciferase. Emission spectra of different luciferase reporter are provided below and form the basis for some homogenous single solution based dual luciferase assays from Targeting Systems

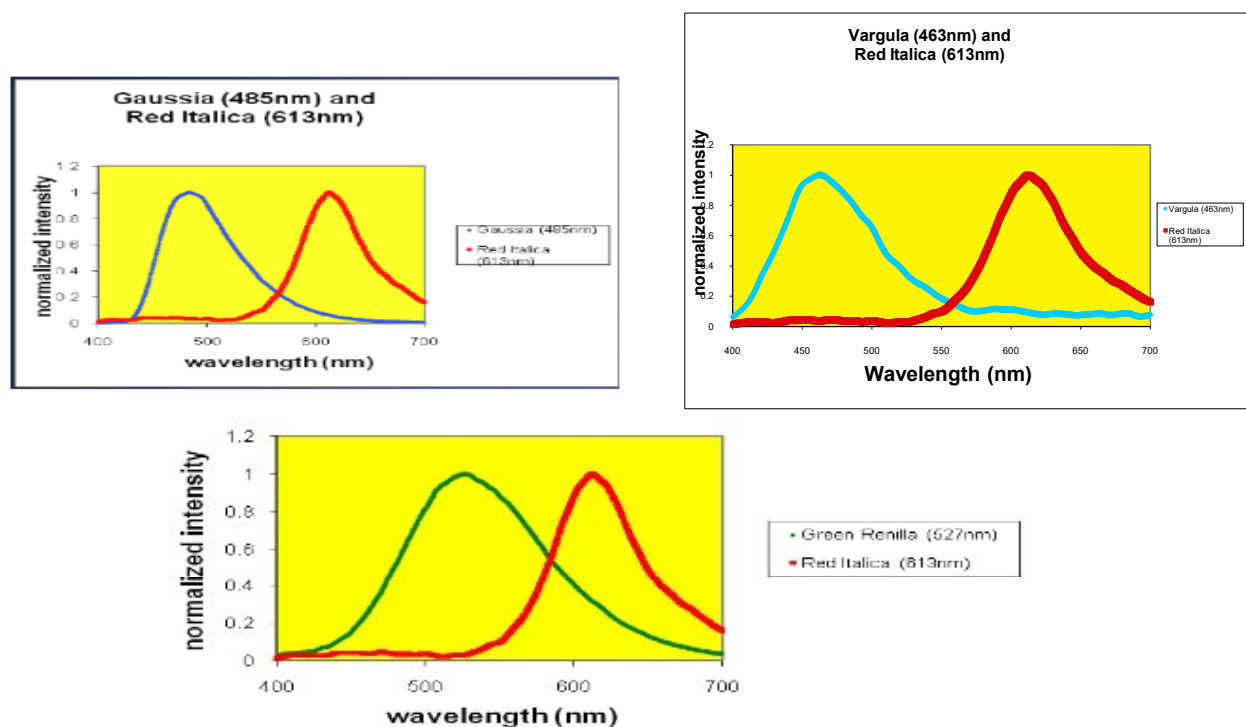


Figure 2: Emission spectra of Green Renilla luciferase and Red Firefly luciferases in samples of transfected cells (lysates or supernatants). The emission spectra were recorded on a Fluorolog-3 spectrofluorometer (Horiba Scientific, Japan) using a liquid nitrogen cooled CCD. The luciferases were assayed by mixing 200 μ l of the sample with the appropriate luciferase assay reagent to obtain spectral profiles. Emission max of Green-emitting Renilla Luciferase is 527 nm; Red italic is 617 nm. (Data courtesy of Justin Rosenberg, Dr Bruce Branchini's lab, Connecticut College, USA)