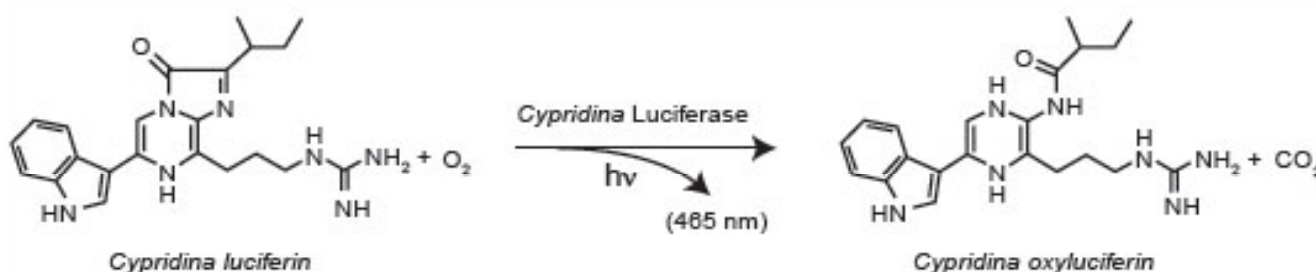


## Expression vectors and assay system for Cypridina (Vargula) Luciferase:

**Another secreted luciferase – Can be multiplexed with Gaussia luciferase, Renilla luciferase and Red-emitting firefly luciferase for dual luciferase or triple luciferase reporter assays**

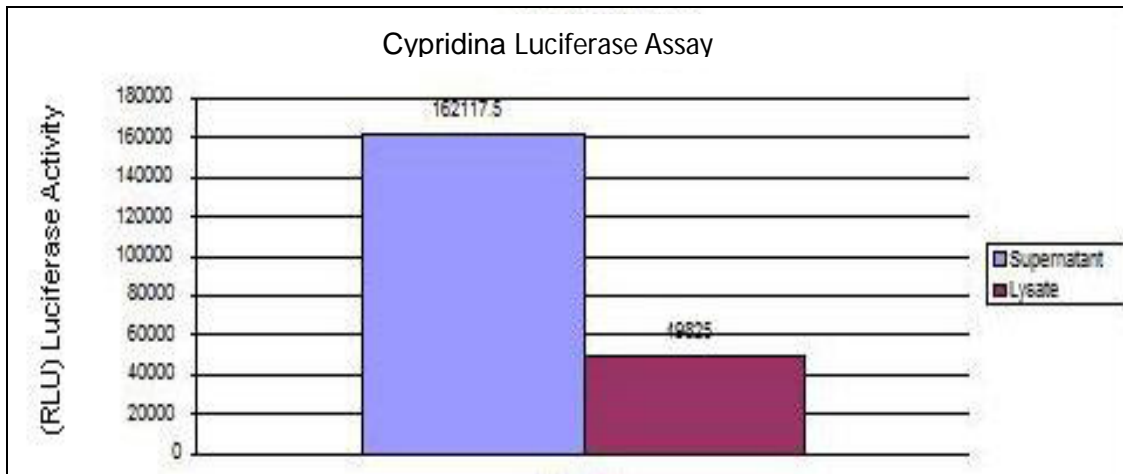
The ostracod *Cypridina (Vargula) Hilgendorfi* when tactilely stimulated ejects a bright-blue luminous secretion into sea water. The luminescence results from an enzyme-substrate reaction in which a small organic molecule, vargulin or cypridina luciferin (Mr, 478), is oxidized by molecular oxygen in a reaction catalyzed by the luciferase (see figure below). The products of the reaction are light, oxyluciferin, and carbon dioxide. The excited-state oxyluciferin bound to luciferase is the emitter in the reaction.



Cypridina luciferase, (formerly known as *Vargula luciferase*) is a secreted luciferase with an emission max of 467 nm. It is one of the brightest known luciferases with the highest turnover number. Unlike Firefly luciferase, Cypridina luciferase does not require ATP. Cypridina Luciferin is different from coelenterazine, the substrate for *Renilla*, *Gaussia* and *Metridia* luciferases. On account of its unique substrate and bright, secreted luciferase activity Cypridina Luciferase is particularly useful in multiplexed assays involving *Gaussia*, *Renilla* or Firefly luciferases. Secreted VLuc 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.

The cDNA for *Vargula luciferase* has been cloned in 1989, and its primary structure has been deduced from the nucleotide sequence (1). *Vargula luciferase* consists of 555- amino acid residues in a single polypeptide chain with two potential N-glycosylation sites (amino acid residues 186-188 and 408-410). The expressed enzyme also possesses a secretion signal, and mammalian cells transfected with cDNA for *Vargula luciferase* secrete the enzyme (2). The activity of luciferase in the culture medium can be readily assayed by mixing an aliquot of the medium with luciferin and measuring the light intensity. Thus, luciferase may be used as a convenient reporter enzyme for studying gene expression in mammalian cells (2,3). Recently Inoue et al, showed that Chinese hamster ovary (CHO) cells transfected with cDNA for *Vargula luciferase* secreted the luciferase, and the secretory process can be monitored in real time from individual cells in the presence of luciferin by using an image-intensification procedure (4). This study demonstrates that *Vargula luciferase* a powerful tool for monitoring gene expression inside a single reporter cell (4). Another interesting application for secreted *Vargula luciferase* is that it is very useful for studying circadian rhythms (3). The *Vargula* reaction is extremely specific, and luciferin does not emit light in an aqueous medium without *Vargula luciferase*. The optimum pH of the reaction is 7.2, and the turnover number (number of molecules of luciferin oxidized per molecule of luciferase) is 1600 per min (4). Luciferase is inhibited by EDTA and EGTA, suggesting that  $Ca^{2+}$  may be involved in its activity (4). The quantum yield is  $0.28 \pm 15\%$  (4).

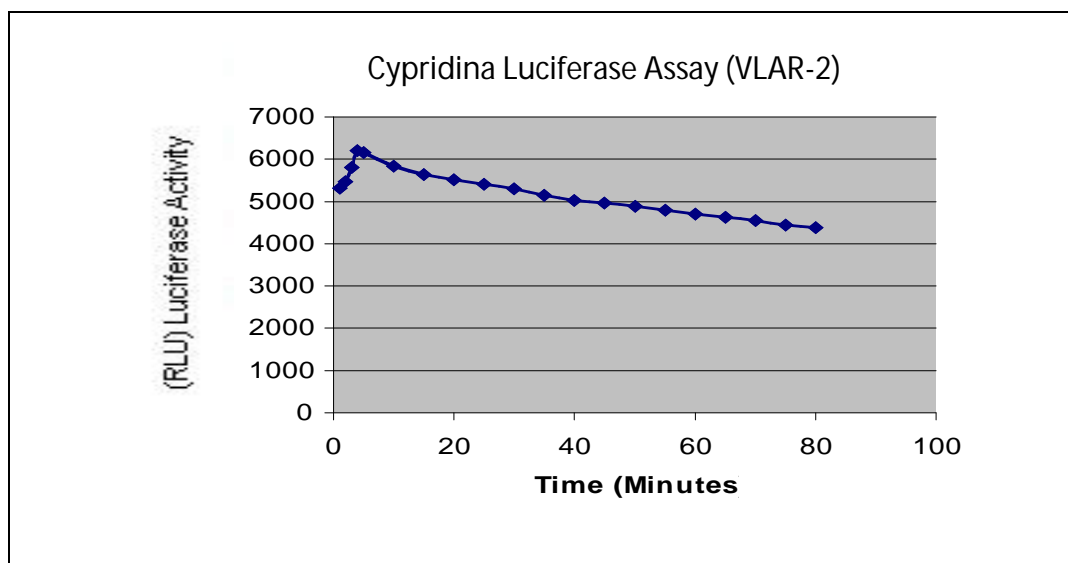
Cypridina luciferase is naturally secreted from cells (Figure 2). Therefore cell-lysis is not necessary for measurement of luciferase activity.



**Figure 2: Intracellular and secreted Cypridina luciferase activity**

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

### Stability of Bioluminescent Signal



**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 expression vector. Samples were assayed using the VLAR-2 (stable version) of the Cypridina luciferase assay reagent. The VLAR-1 reagent has an excellent stability profile for the first 10 mins and can be used for regular assays but for HTS (high throughput screening), we recommend the VLAR-2 reagent.

## Advantages of using Cypridina (Vargula) luciferase

- ✚ *Cypridina* Luciferase is secreted from cells by virtue of its natural signal peptide and its luminescence can be measured from the supernatant of transfected cells. Therefore, cell lysis is not necessary and multiple time points can be assayed from the same group of transfected cells.
- ✚ *Using the VLAR-2 version of the assay reagent*, the *Cypridina* luciferase reaction shows excellent stability of the bioluminescent signal over 45 mins making it suitable for high throughput screening applications
- ✚ Secreted *Cypridina* luciferase 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 (Figure 2).
- ✚ The *Cypridina* luciferase assay is very sensitive, allowing detection of very small amounts of *Cypridina* Luciferase activity
- ✚ Secreted *Cypridina* luciferase is thermally stable at 55°C which is the typical inactivation temperature of most viruses.
- ✚ The fact that *Cypridina* luciferase utilizes a distinct substrate- *Cypridina* luciferin, which is different from the substrate for Renilla, Gaussia or firefly luciferases makes it well suited for multiplexed luciferase assays. Several multiplexing options are available using *Cypridina* luciferase in dual and triple luciferase reporter assays (secreted or intracellular)
- ✚ Options are available wherein *Cypridina* luciferase can be assayed with a red-emitting firefly luciferase (from *Luciola Italica*) using a single assay reagent (DLAR-3) and spectrally resolving the two luciferase activities using appropriate filters. This significantly increases speed of assay and decreases screening costs.
- ✚ *Cypridina* luciferase is also very useful for in vivo imaging applications and can be used to image single cells.

## Cypridina Luciferase Assay Protocol

### 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. Store at 4°C.

**3. VLAR (Cypridina luciferase assay buffer)**

The VLAR buffer (Cypridina luciferase assay buffer) is provided in a 50ml bottle. Store at 4°C

**4. Cypridina stabilizer (dark brown color) 100X concentrated**

Store at -80 or -20°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 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 with stabilizer:**

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 4 ml of VLAR buffer just before use. Make sure both solutions are thawed to room temperature prior to mixing. **Please make sure to add the stabilizer (40 ul per 4 ml of VLAR buffer) just before you are ready to assay.**

### Assay Protocol:

1. Pipette 5-20 ul of sample into each assay well or luminometer tube
2. Add 40 ul of VLAR buffer with stabilizer
3. Add 20 ul of diluted Cypridina luciferin substrate.
4. Mix well and assay

**Note:** Cypridina luciferase is a secreted enzyme and most of the activity is secreted (75-80%) in the supernatant. **We recommend using OptiMEM 1 or complete media with low serum content (3% or less) as this reduces the background of the assay.** We recommend assaying samples at 48 hours 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) from Targeting Systems (see protocol below), as it is compatible with 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, 800ul for a 6-well dish, and 3 ml for a 10 cm dish).
4. Shake for 20 min at 400rpm on an orbital shaker (room temperature).
5. Mix 5-20  $\mu$ l of luciferase containing sample or cell lysate with 100  $\mu$ l 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.*

### 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) for all samples as well as for priming the injector as suggested by the manufacturer. Protect this solution from light.

**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 at [info@targetingsystems.com](mailto:info@targetingsystems.com) or [targetingsystems@gmail.com](mailto:targetingsystems@gmail.com).

Please check our website [www.targetingsystems.net](http://www.targetingsystems.net) for novel luciferase-based multiplexed assays.

### References

1. Thompson, E. M., Nagata, S., and Tsuji, F. I. (1990) Vargula hilgendorffii 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.

## Multiplexing Options (Dual and Triple luciferase reportr assays:

### DLAR-3 :

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

### DLAR-4 :

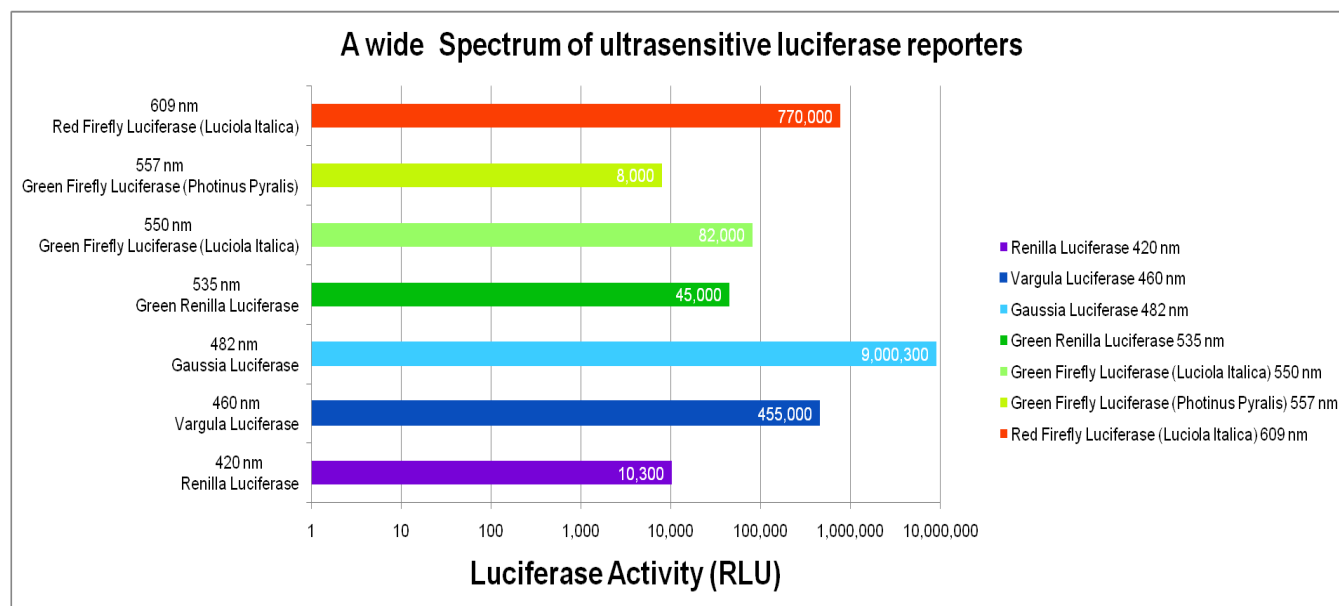
A dual assay reagent based on a secreted (or intracellular) Cypridina luciferase and a secreted (or intracellular) Gaussia Princeps luciferase. This assay is compatible with high throughput applications.

### 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

### TLAR-2 :

A single solution-based triple luciferase reporter assay involving Gaussia luciferase multiplexed with Cypridina 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



**Figure 4: Luciferase reporters offered by Targeting Systems:**

The Photinus luciferase used in this experiment was the PGL3 series Photinus luciferase subcloned under control of the CMV promoter





