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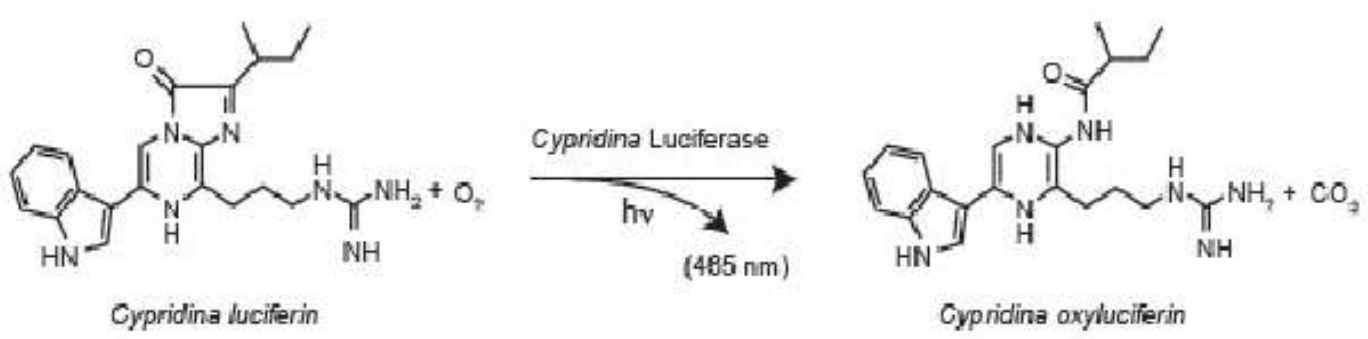
An assay system for Vargula (Cypridina) luciferase

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

Novel ultrasensitive secretory and intracellular luciferase reporter for drug discovery applications:

The small (-3-mm long) marine ostracod crustacean, Vargula (formerly Cypridina) hilgendorfii, 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 .



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 Ca2+ may be involved in its activity (4). The quantum yield is 0.28 ± 15% (4).

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Advantages of vargula luciferase as a reporter

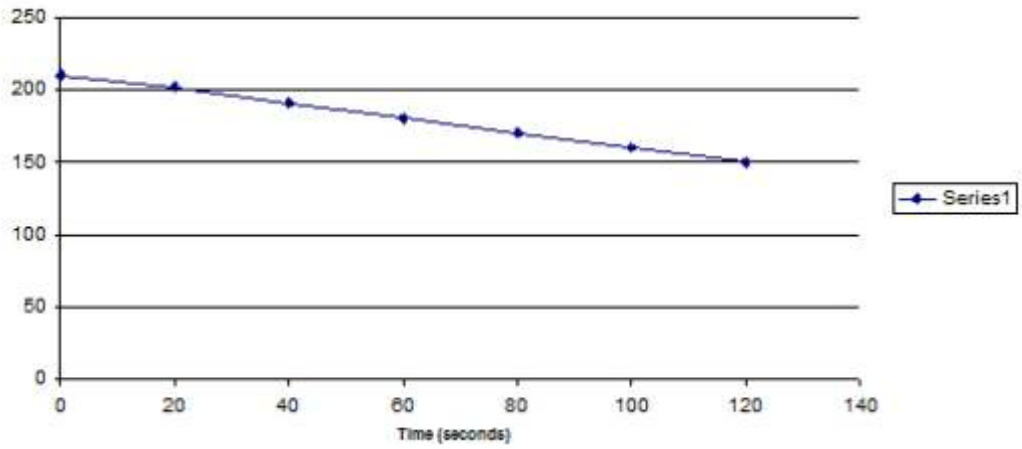
Increased sensitivity: Vargula luciferase is significantly brighter than firefly luciferase (10-20-fold brighter)
It can be used for live cell imaging of individual cells
Gene expression in transiently transfected cells can be monitored over a greater time period (120 hrs) than in case of firefly luciferase transfected cells (72 hrs)
Available both as a secreted reporter (modified native vargula luciferase) or as an intracellular reporter (vargula luciferase modified with an endoplasmic reticulum retention signal so that it si retained inside the cells
Can be used to monitor gene expression in real time since the substrate can diffuse across living cells.
Vargula luciferase emtis blue light (emission maxima at 462 nm) and is therefore very useful in multiplexed assays in combination with green- emitting renilla or fierefly luciferase (emission maxima 547-550 nm) or a red-emitting firefly luciferase (emission maxima 609 nm) . I can also be used as aa reporter gnee in combination with Gaussia luciferase which utilizes coelenterazine as a substrate. Single solution multiple luciferase reporter assays offer significant saving in cost and screening time.

References:

1) Thompson, E. M., Nagata, S., and Tsuji, F. I. (1989) Cloning and expression of the cDNA from the ostracod vargula hilgendorfi Proc. Natl. Acad. Sci. U. S. A. 86, 6567–6571
2) Thompson, E. M., Nagata, S., and Tsuji, F. I. (1990) Gene (Amst.) 96, 257–262
3) New reporter system for Per1 and Bmal1 expressions revealed self-sustained circadian rhythms in peripheral tissues (2006) Shin-ya Nishide, Sato Honma, Yoshihiro Nakajima, Masaaki Ikeda, Kenkichi Baba, Yoshihiro Ohmiya, and Ken-ichi Honma Genes Cells, Oct 2006; 11: 1173 - 1182.
4) S Inouye, Y Ohmiya, Y Toya, and FI Tsuji (1992) Imaging of Luciferase Secretion from Transformed Chinese Hamster Ovary Cells PNAS, Oct 1992; 89: 9584 - 9587

Figure 1: Stability of the vargula luciferase bioluminescent signal.

Figure 1: 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 expressionvector. Using the VALR-1 reagent. A more stable signal is obtained using VLAR-2 assay reagent.



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Intracellular and secreted Vargula luciferase activity

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

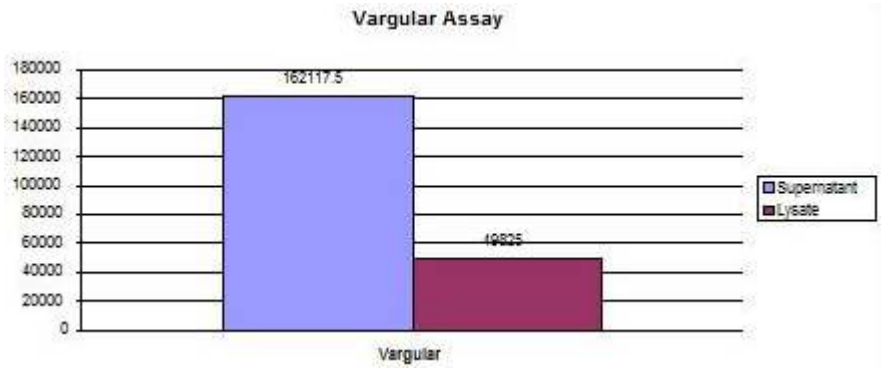

Figure 2: Intracellular and secreted Cypridina luciferase activity
Luciferase activity in cell supernatants and cell lysates of cell transfected with either a plasmid vector expressing secreted vargula luicferase

Catalog No.	Size	Description	Price
		Cypridina Luciferase Expression Vectors:	
CMV-VargLuc	VL-001	Expresses Cyprdina (Vargula) luciferase under control of the CMV promoter	$ 299.00
pBasic-VargLuc	VL-002	Promoterless vector expressing Cypridina Luciferase	$ 299.00
		Cypridina Luciferase (single) Assay Reagents	
VLAR-1	1000 assays	Cypridina (Vargula) Luciferase Assay Reagent	$ 400.00
VLAR-2	1000 assays	Cypridina Luciferase Assay reagent (more stable version)	$ 440.00
		Cypridina Luciferase Dual Assay reagents	
DLAR-3	1000 assays	Cypridina-Red Firefly Luciferase	$ 850.00
DLAR-4	1000 assays	Cypridina-Gaussia Luciferase	$ 900.00
DLAR-5	1000 assays	Cypridina-Renilla Luciferase	$ 900.00
		Cypridina Luciferase Tripke Reporter Assays	
TLAR-1	1000 assays	Cypridina-Green Renilla-Red Firefly Luciferse Assay Reagent	$ 1000
TLAR-2	1000 assays	Cypridina Luciferase Gaussia Luciferase, red Firefly Luciferase Assay reagent	$ 1000

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Assay Protocol

We recoomend assaying supernatants 48-72 hours after transfection
Mix 20 ul of Cell supernatant (DMEM or DMEM with 5% serum) with 50 ul of the VLAR assay buffer and 31.5 ul of the thawed vargulin substrate . Mix well and read in the luminometer (1-20 sec integration)

Related Product: A wide spectrum of ultra-sensitive luciferase reporters
Single solution-based multiplexed lucifferase assays using Vargula luciferase: Please enquire 1-866-620-4018
Vargula Luciferase vector Sequences : Please refer to technical resources
For additional information
Coding sequence of vargula luciferase is presented on the following page.
The plasmid maps of the pCMV-Gluc and the pGluc-Basic vectors can be obtained from our website at the following links
http://targetingsystems.net/ts_novel_luciferase_assay_system.htm

NOTE :
The composition of the Vargula luciferase assay reagent and multiplexed luciferase assays using vargula are covered under pending patents. For more information please contact Mr Wesley Ames: 760-471-9620 or enquire at 1-866-620-4018

Construction of pCMV-VargLuc and pBasic-VargLuc expression vectors: The human codon optimized sequence of Vargula Hilgendorfi was subcloend into the BamH1 and Xba 1 sites of the PCMV-GLuc and the pGLuc-Basic vactors so that the Gaussia luciferase gene was replaced with the Vargual lcuiferase gene. The sequences of the pCMV-GLuc and the PGluc –Basic vectors can be obtained from our website. The sequence encoding Vargula Hilgendorfi is presented below:

Assay Protocols and Plasmid Maps or sequences: Detailed assay protocols, plasmid maps and sequences are provided with the Cypridina luciferase assay reagents and can also be downloaded form the technical resources section of our website www.targetignsystems.net

Contents and Storage:

Each Cypridina luciferase assay kit (VALSR-1 or VLAR-2) 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) This can be stored at 4 ° C.
3. CLAR (Cypridina luciferase assay buffer). The VLAR buffer (Cypridina luciferase assay buffer) is provided in a 50 ml bottle. This can be stored at 4 ° C .
4. VLAR-2 also contains a stabilizer (store at – 20 ° C.

All plasmids should be stored at – 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