

VLAR-2: Assay reagent for Cypridina Luciferase for assay of Cypridina luciferase from Cypridina Noctiluca or Vargula Hilgendorfi

Catalog no.	Size	Description	Price
VLAR-2	1000 assays	Cypridina luciferase assay reagent	\$440.00

Product Description:

Cypridina luciferase, (formerly known as Vargula luciferase) from the marine ostracod Vargula Hilgendorfi or Cypridina Noctilucus is a secreted luciferase with an emission max of 460 nm. It is one of the brightest known luciferases with the highest turnover number. Unlike Firefly luciferase, Cypridina luciferase does not require ATP and catalyses oxidation of it's unique substrate Cypridina luciferin as described below (Figure 1).

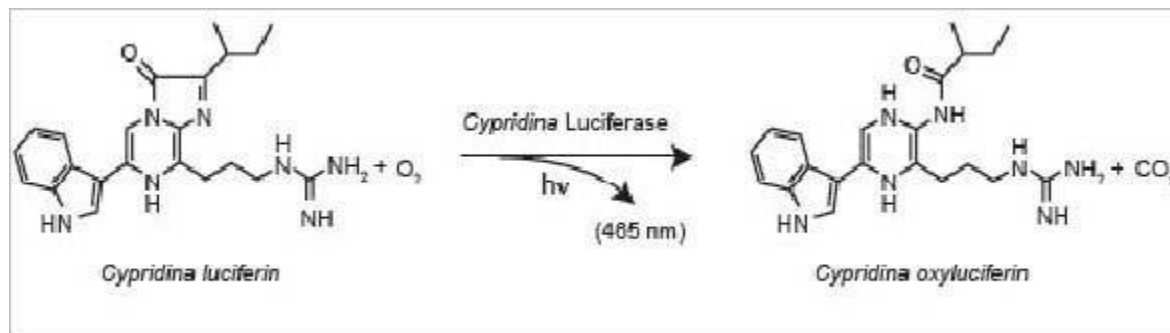


Figure 1: The photochemical reaction catalyzed by *Cypridina* Luciferase.

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

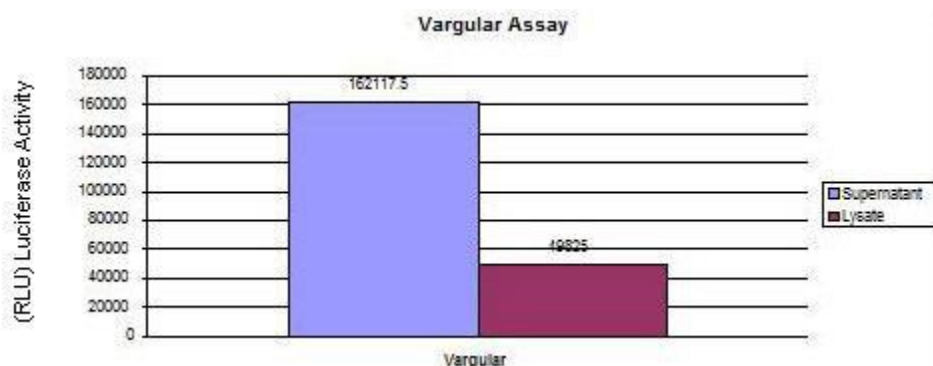


Figure 2: Cypridina luciferase activity in cell supernatants and cell lysates of cell transfected with either a plasmid vector expressing secreted Cypridina luciferase. In cells transfected with the native Cypridina luciferase, 80% of activity is secreted into the cell supernatant and only 20% is cell-associated.

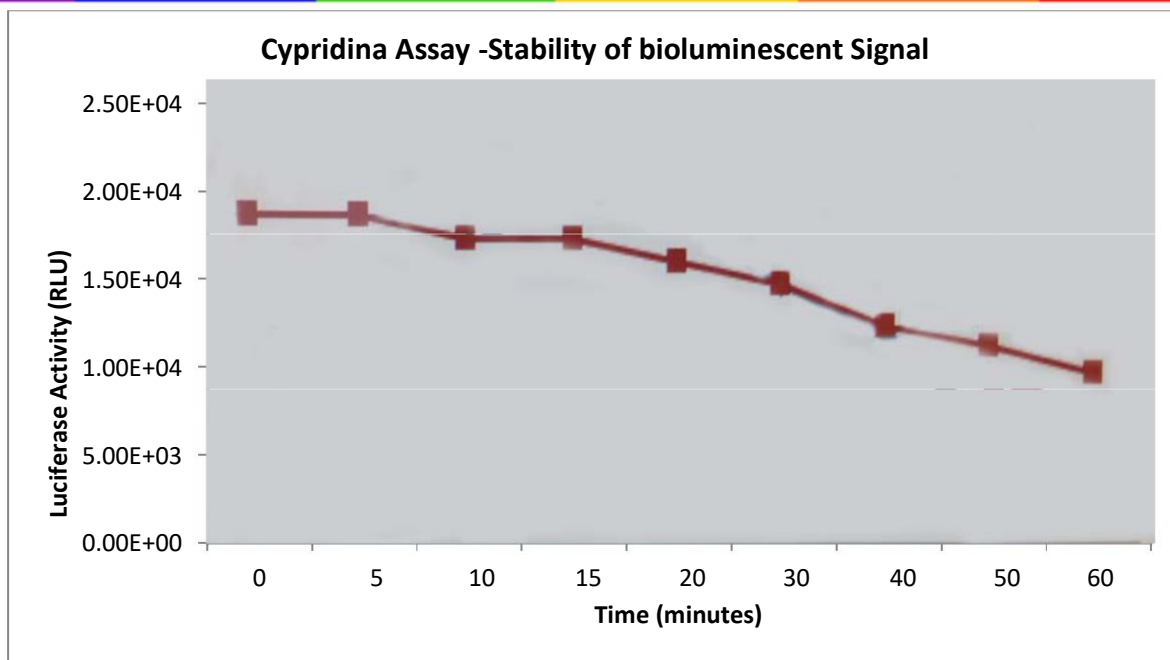


Figure 3: Kinetics of light emission. The stability of the bioluminescent signal of Cypridina Luciferase was assessed using supernatants from HEK 293 cells transiently transfected with the pCMV-VLuc expression vector.

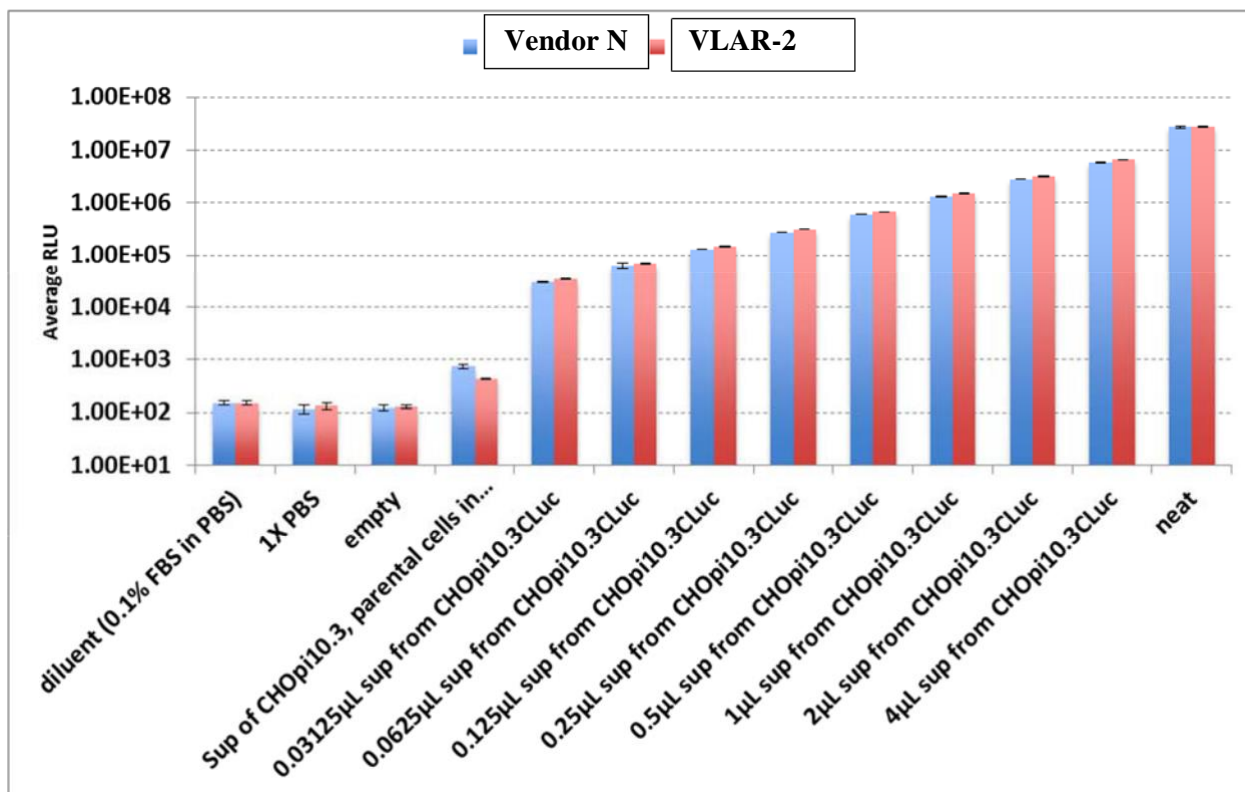


Figure 4: Comparison of Cypridina (Vargula) luciferase assay reagent (VLAR) from Targeting Systems with Cypridina luciferase assay reagent from vendor N. Assayed 20µL of sample, 50µL injection, 2 seconds of delay and 2 seconds of integration on Mithras LB940 (Berthold Technologies). CHOpi10.CLuc cell line is a stable cell

line harboring a single copy of cluc gene expresses CLuc. Eight 2-fold serial dilutions were made from the growth medium of the stable cell line using a diluent made up of 0.1% FBS in 1X PBS. The CHOp10.3 is the parental cell line which does not express CLuc

CYPRIDINA LUCIFERASE ASSAY PROTOCOL::

Contents and Storage:

Each kit contains the following:

1. Cypridina luciferin substrate (100 X). Store at – 80 ° C.
2. Cypridina substrate dilution buffer (50 ml) (Provided in a brown bottle). This can be stored at 4 ° 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.

ASSAY PROTOCOLS:

Standard Assay Protocol I (Luminometers without injectors)

1. Prepare the Cypridina luciferase assay solution (e.g. 100 samples) by adding 50 µl of the 100X Cypridina luciferin Substrate to 5 ml of Cypridina luciferase substrate dilution buffer immediately before performing the assay.
2. Mix well by inverting the tube several times (Do not vortex).
3. Set the luminometer for 2–10 seconds of integration.
4. Pipet samples* (5–20 µl per well) into a 96-well white (opaque) or black plate, or a luminometer tube.
5. Add the Cypridina luciferase assay reagent (50 µl) to a sample and promptly measure the luminescence.
6. Repeat Step 5 for all samples.

We recommend using OptiMEM 1 or complete media with low serum content (3 percent or less) as this reduces the background of the assay. We recommend assaying samples at 48 hrs post transfection. In order to measure Cypridina luciferase activity in the Cell lysates we recommend using the 5X Cell Lysis Reagent (catalog no. 5X CLR1, see protocol below) from Targeting Systems, as this is compatible for assaying 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, 800 ul for a 6-well dish and 3 ml for a 10 cm 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 50 ul of the Cypridina luciferase assay reagent and read immediately in the luminometer as described above
6. All assay reagents should be close to room temperature at the time of assay.

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.