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

Catalog no.	Size	Description	Price
VLAR-2	1000 assays	Cypridina luciferase assay reagent	$440

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 lcufierase does not require ATP and catalyses oxidation of it's unique substrate Cypridina luciferin as described below (Figure 1).

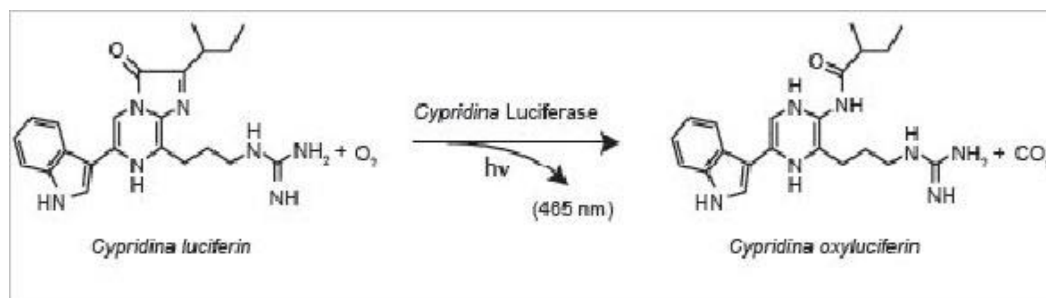

Figure 1: The photochemical reaction catalyzed by *Cypridina* Luciferase.

Cypridina Luciferin is different form coelenterazine, the substrate for Renilla, Gaussia and Metridia luciferses. On account of it's unique substrate and bright, secreted luciferase activity Cypridina Luciferase is particularly useful in multiplexed assays involving Gaussia, Renilla or Firefly luciferses. 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 form cells (Figure 2). Therefore cell lysis is not necessary for measurement of luciferase activity.

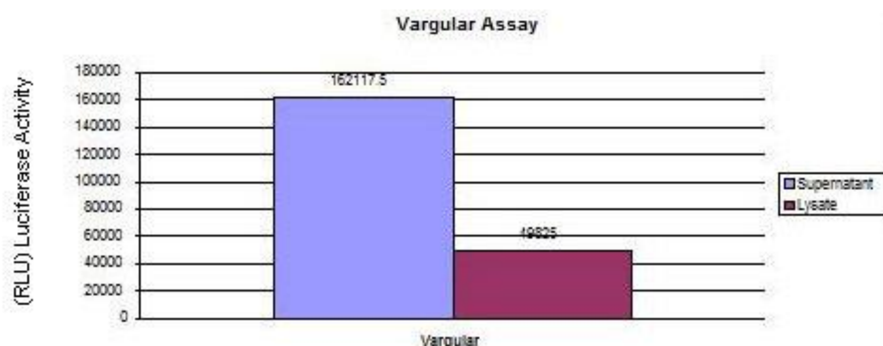

Figure 2: Luciferase activity in supernatants and lysates of cells transfected with a plasmid vector expressing Cypridina luciferase. In cells transfected with the native Cypridina luciferase, 80% of activity is secreted into the cell supernatant and only 20% is cell–associated.

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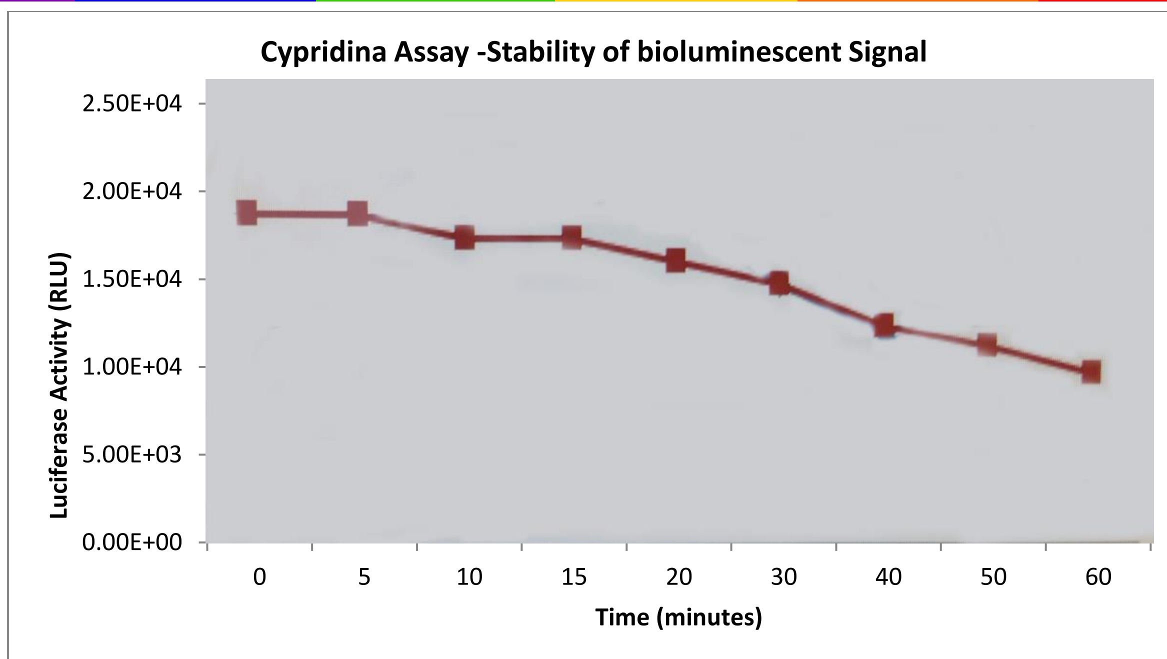

Figure 3: Kinetics of light emission. The stability of the bioluminescent signal of Cypridina Luciferase was assessed using supernatants from HEK 293 cells transiently transfected with the pCMV-VLuc expressionvector.

SUPPORTING COMPARISION DATA

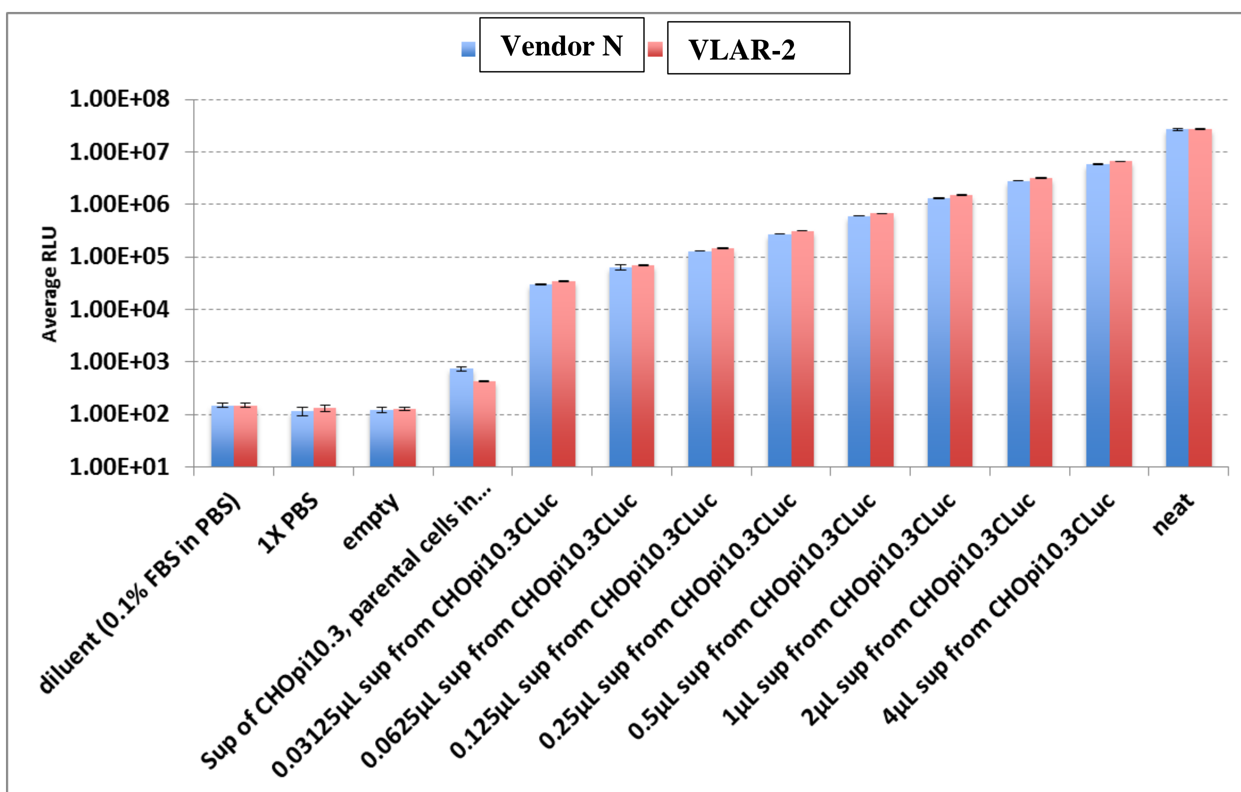

Figure 4: Comparision of Cypridina (Vargula) luciferase assay reagent (VLAR) from Targeting Systems with Cypridina luciferase assay reagent from vendor N. Assayed 20μL of sample, 50μL injection, 2

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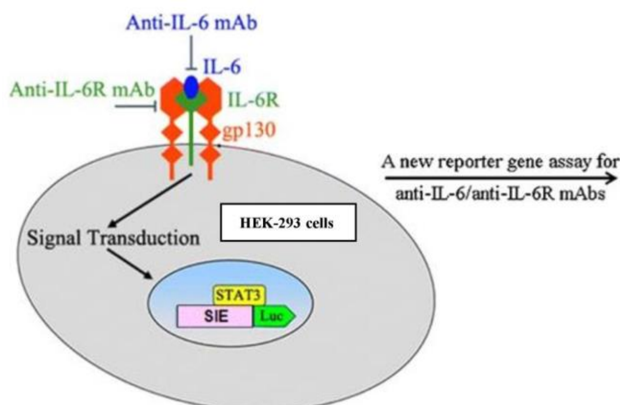
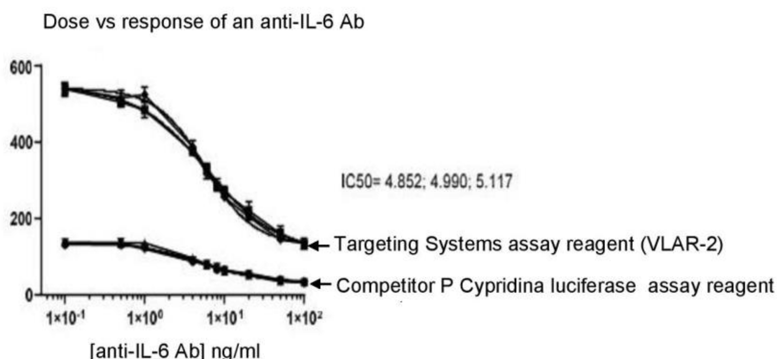
seconds of delay and 2 seconds of integration on Mithras LB940 (Berthold Technologies). CHOpi10.CLuc cell line is a stable cell line harboring a single copy of cluc gene expresses CLuc. Eight 2-fold serial dilutions were made from the growth medium of the stable cell line using a diluent made up of 0.1% FBS in 1X PBS. The CHOpi10.3 is the parental cell line which does not express CLuc





Mechanism sketch of the reporter gene assay for bioactivity determination of anti-IL6 or anti IL6R antibodies

Figure 5: Evaluation of inhibitory activity of an anti-IL-6 antibody using a Cypridina luciferase assay from Targeting Systems (VLAR-2) or a competietor (P). HEK-293 cells responsive to IL-6 cells were generated by stably introducing the human IL-6R gene into IL6R deficient HEK-293 cells. In order to detect activation of the IL-6 pathway they were modified with an ultrasensivite reporter gene expressing secreted Cypridina luciferase under control of a modified minimal CMV promoter fused to 6 repeats of STAT-3 binding sites. In addition a selection marker and a second secreted Luciferase for normalization were introduced under a constitutive active promoter. Upon IL-6 stimulation, HEK-Response IL-6 cells trigger the activation of STAT3 and the subsequent secretion of Cypridina luciferase. Levels of STAT3-induced Cypridina luciferase or inhibitory activity of an anti IL-6 anitibody can be readily monitored by measuring Cypridina luciferase activity. Figure 2 shows a comparison of the CYrpdiian luciferase signal measured using the Cypridia luciferase assay reagent for Targeting Systems (VLAR-2) or the Cypridina luciferase assay reagent from a competeitor P .
Data courtesy of Dr Matthias Hamdorf, Terasaki Insttitute, Los Angeles, CA

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:

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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 cell.s (50 ul for 96-well, 300 ul for a 12-well, 800 ul for a 6-well dish and 3 mll 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 citing the use of the Gaussia and Cypridna luciferase assay reagents from Targeting Systems

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