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UltraBrite™ Cypridina-Gaussia dual luciferase assay reagent

Ultrasensitive, sequential detection of Cypridina luciferase (CLuc) and Gaussia luciferase (GLuc)

Catalog No.	Size	Description	Price
DLAR-4 SG-1000	1000 reacns	***UltraBrite™* Cypridina-Gaussia dual luciferase assay reagent**	$850
DLAR-4 SG-500	500 reacns	***UltraBrite™* Cypridina-Gaussia dual luciferase assay reagent**	$450

Assay background and Principle: The ***UltraBrite™* Cypridina-Gaussia dual luciferase assay** is a rapid ultrasensitve dual luciferase assay for sequential detection of Cypridina luciferase (CLuc) and Gaussia luciferase (GLuc) in samples of supernatants or lysates from transfected cells. The assay depends on quenching the bioluminescence of CLuc with inhibitors present in the GLuc assay buffer buffer thus allowing measurement of both luciferases from the same sample saving plates and assay time. The assay is far more sensitive than Firefly-Renilla dual luciferase assays because the brightness of the CLuc and GLuc reporters is 1000-times and 20-times brighter than these early generation Luc reporters. Both reporters are secreted, so the assay does not require cell lysis and assays can be carried out sequentially over several days using the same cell population. Improved assay sensitivity and the ability to study gene expression in real time make this assay particularly attractive for high throughput screening. The substrate for CLuc (Cypridina luciferin) is different form the substrate for GLuc (coelenterazine) so there is no cross reactivity.

Advantages:

- More sensitive than Fireflc-Renilla dual luciferase assays because the brightness of the CLuc and GLuc reporters is 1000-times and 20-times brighter than these early generation Luc reporters.
- Both the CLuc and GLuc reporters are secreted, so there is no need for cell lysis and assays can be carried out sequentially over several days using the same cell population.
- Allows study of weak promoters, low-level gene expression in cells that transfect poorly
- Samples can be stored at -20°C for months without loss of activity.
- Cohort of samples taken from the same culture dish/well at different times can be assayed together

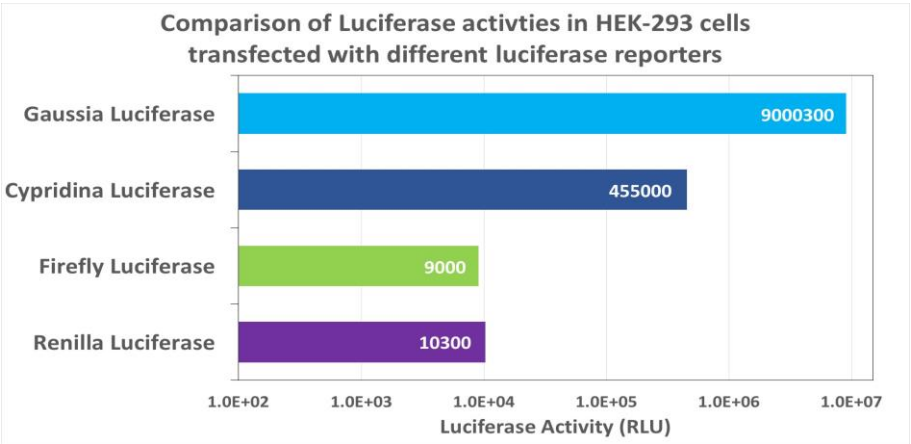

Figure 1: Comparision of luciferase reporter activities: HEK-293 cells were grown in DMEM with 3% serum) and transfected with plasmids expressing (Gaussia, Cypridina, Firefly or Renilla luciferases under control fo the CMV promoter using the Targefect-F1 reagent Targeting Systems) as per the manufacturer's protocols. The expression vectors pCMV-GLuc, pCMV-CypLuc, pCMV-FLuc and pCMV-GrRenLuc were also from Targeting Systems. Total luciferase activity in tansfected cell supernatants (GLuc or CLuc) or lysates (FLuc and RLuc) was measured 48 hrs post transfection using the GAR1 reagent (GLuc assay), VLAR-2 reagent (CLuc assay), FLAR-1 reagent (FLuc assay) or RLAR-1 reagents (Renilla assay) from Targeting Systems, El Cajon, CA.

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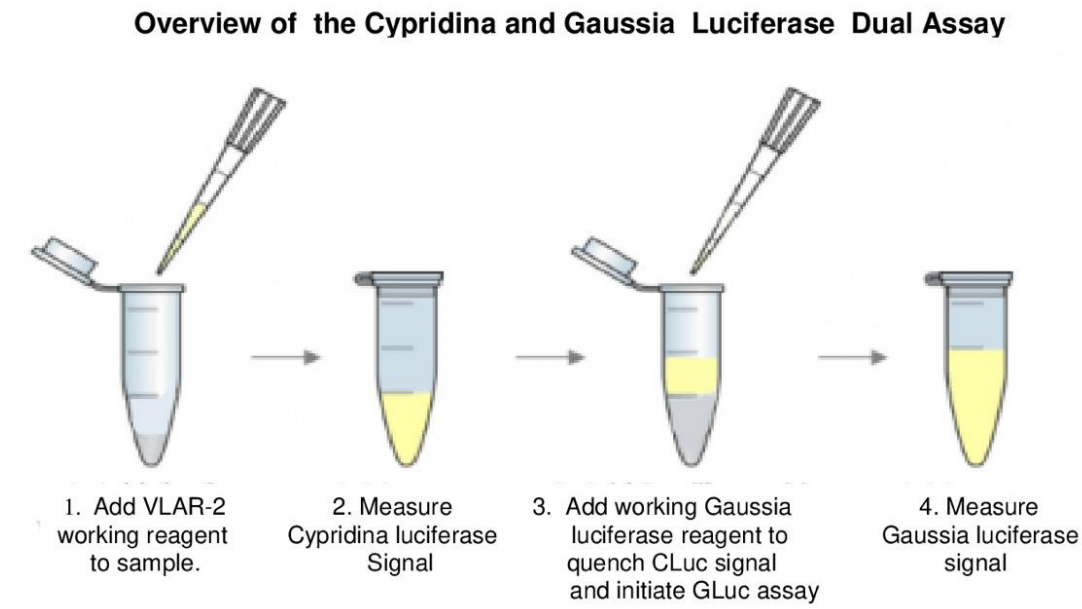
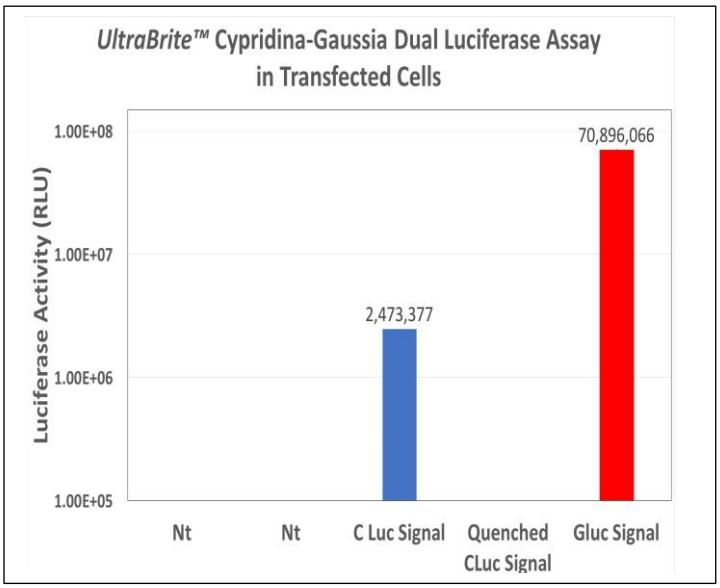


Figure 2: Overview of the Cypridina-Gaussia luciferase *Quench & Glo* ™ protocol. The assay can be performed in cell lysates supernatants or cell lysates (prepared using the cell lysis reagent (5XCLR-1) from Targeting Systems. This cell lysis reagent is designed for compatibility with the GLuc and CLuc reporters



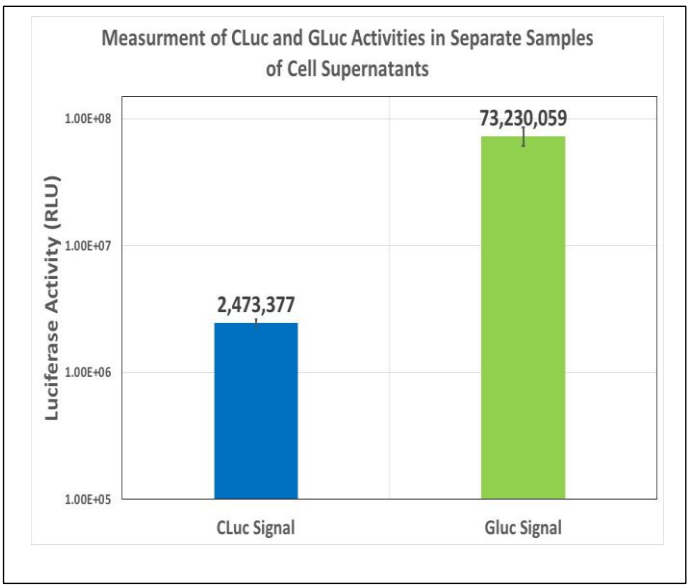


Figure 3A: Sequential detection of Cypridina luciferase and Gaussia luciferase in cell supernatants using the *UltraBrite* ™ Cypridina-Gaussia dual luciferase assay reagent: Aliquots of supernatants (10 ul) from transfected HEK 293 cells were assayed with 50µL of VLAR2 reagent to measure CLuc activity. After 2 minutes 50µL of the working Gaussia lcuiferase assay component (GAR *Quench & Glow*™ Reagent)t was added to quench the Cypridina luciferase activity and measure Gaussia luciferase activity. Nt refers to untransfected control cells assayed with either VLAR-2 or the GAR Quench & Gloreagent (RLU were 200 and 400 respectively. The quenched GLuc signal was 12000 RLU. Data shows mean of 5 determinations along with the standard deviations

Figure 3B: Measurement of Cypridina and Gaussia luciferase activities in separate samples of cell supernatnats:. Aliquots of supernatants (10 ul) from transfected HEK 293 cells were assayed with either 50µL of VLAR2 reagent to measure CLuc activity or with 50 ul of VLAR-2 buffer (no CLuc substrate) plus 50 ul of GAR ***Quench and Glo*** ™ reagent to measure Gluc acitivty . Data shows mean of 5 determinations along with the standard deviations

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Stability of the CLuc and GLuc bioluminescent signals using the ***UltraBrite™*** **Cypridina-Gaussia dual luciferase assay reagent**

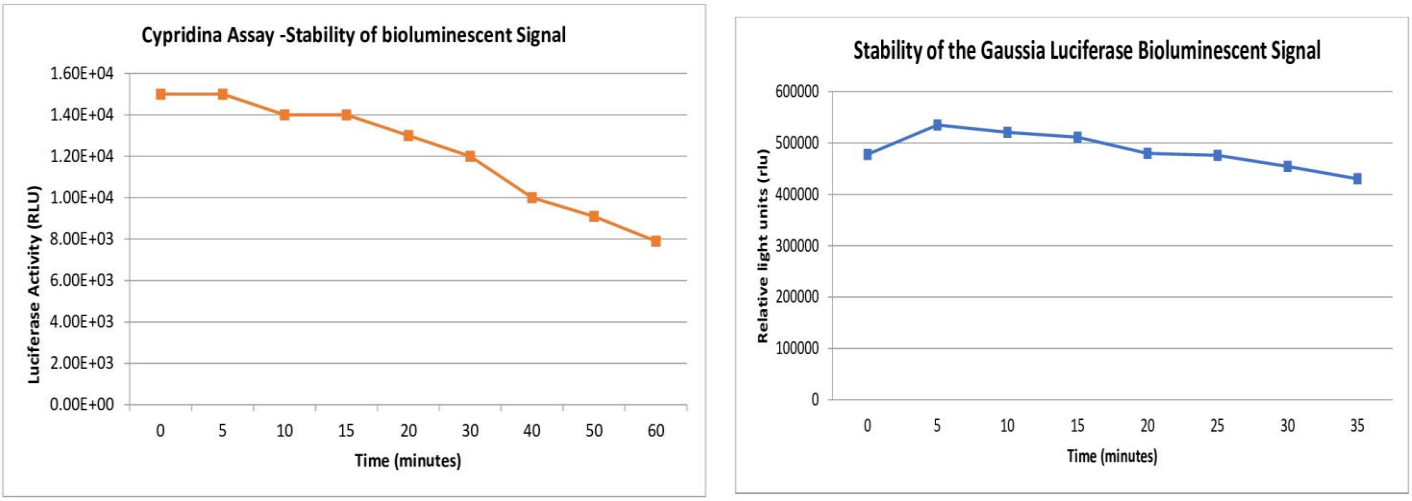

Figure 5: Kinetics of light emission: The stability of the CLuc and GLuc bioluminescent signals was assessed by assaying supernatant media from HEK-293 cells transientl transfected with the VLAR-2 or GAR ***Quench and Glo ™*** reagent components of the ***UltraBrite™*** **Cypridina-Gaussia dual luciferase assay reagent**. Data shown is mean of quadruplicate determinations.

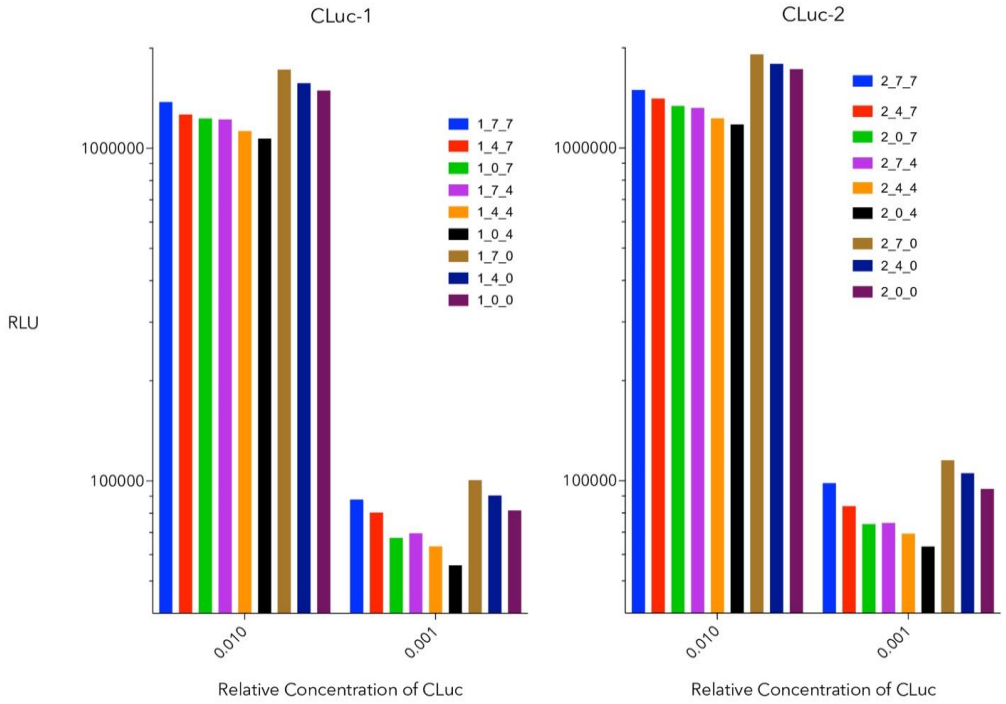

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Figure 7: A mini-aging experiment looking at stability of two CLuc samples and the VLAR2 reagent over a one week period. The samples shown in the graph are labeled as follows: X_Y_Z where X is the sample number (1 or 2), Y is the number of days the CLuc sample was left at room temperature (7, 4 or 0), and Z is the number of days the VLAR2 reagent was left at room temperature (7, 4 or 0). In all cases this was added to the VLAR2 reagent from the same freshly thawed 100x tube. The Club substrate was tested

Data courtesy of Dr. Nigel Killeen. Atum Newark, California.

COMPONENTS AND RAPID PROTOCOL:

Component 1

- **VLAR-2 Cypridina Substrate dilution Buffer-Store at 4 º C**
- **100X Cypridina luciferin- Store at -80 º C**

Component 2

- **GAR *Quench and Glo™*** Buffer
- **100X GAR substrate**

Working Reagent Preparation: Prepare working reagents by diluting 100X substrates with the respective assay dilution buffers just before use and use within 90 mins. All assay reagents should be close to room temperature at the time of assay. Samples can be stored at -80ºC or -20^{0}C for long term storage as assayed later. We have found that cell media supernatants containing CLuc and Glcu can be stored at -80 ºC for over a year without loss of activity

RAPID ASSAY PROTOCOL:

1. Pipette 5-10µl of cell media supernatant into tubes or wells of a microtiter plate (white-walled plates are preferred). (The assay can also be performed in micrrotubes using a tube luminometer)
2. Add 50µl of the working VLAR-2 reagent from the *UltraBrite ™* Cypridina-Gaussia dual luciferase assay reagent and measure CLuc activity in a microplate luminometer (we used a Berthold Impulse 3 microplate luminometer for our studies and use an integration time of 2 sec//well for this assay. The working VLAR-2 reagent is prepared by dilution the 100X Cyripdina luciferin to 5 ml using the VLAR-2 substrate dilution buffer
3. Wait 1-2 min (usually this is not required as it takes about several minutes to read a 96-well plate)
4. Add 50µl of the working GAR *Quench and Glo™.* And measure GLuc activity by luminometry. The working GAR *Quench and Glo™* reagent is prepared by dilution the 100X GAR substrate to 5 ml using the GAR *Quench and Glo™* dilution buffer

Assay performed using cell lysates:
NOTE: This assay can also be performed on cell lysates. If performing an assay on cell lysates, then first lyse the cells using the cell lysis buffer from Targeting Systems (catalog # 5X CLR-1) following the instructions below and use 5-10µl cell lysate instead of cell supernatant and follow steps 1-4

Measurement of intracellular luciferase activity: Lyse cells using our lysis buffer (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.

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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 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 µl of the working luciferase assay reagent and read immediately in the luminometer.
6 All assay reagents should be close to room temperature at the time of assay.

OTHER CONSIDERATIONS:
It is preferable to use low-serum media because high serum concentrations can interfere with the assay. We have used OptiMEM with low serum concentrations (up to 3%) for this assay.

Since the GLuc and CLuc signals are very bright we recommend using small volumes of sample (preferably under 10µl) and no more than 25µl per sample.

The Gaussia luciferase reagent should be prepared fresh just before use. Once prepared, the working GAR reagent should be used within 90 mins.

SEE DETAILED PRODUCT PROTOCOLS AT THE FOLLOWING LINK ON OUR WEBSITE
www.targetingsystems.net/product-protocols.php

FOR ADDITIONAL INFORMATION:
Email us at info@targetingsystems.net or call us at 1-888-818-2446; 619 562 1518