targetingsystems

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UltraBrite™ Cypridina-Gaussia dual luciferase assay reagent

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

Catalog No.	Size	Description	Price
DLAR-4 SG-100	1000 reacns	***UltraBrite™* Cypridina-Gaussia dual luciferase assay reagent**	$850
DLAR-4 SG-100	200 reacns	***UltraBrite™* Cypridina-Gaussia dual luciferase assay reagent**	$300

Assay background and Principle: The ***UltraBrite™* Cypridina-Gaussia dual luciferase assay** detects Cypridina luciferase (formerly known as Vargula luciferase) which utilizes the Cypridna luciferin substrate nad Gaussia luciferase which utilizes a different substrate ,coelenterazine. This assay is based on the principle that the bioluminescence of CLuc can be quenched by inhibitors present in the GLuc assay buffer thus allowing measurement of both luciferase form the same sample saving paltes 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 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. Improved assay sensitivity plus the ability to study gene expression in real time makes this assay particularly attractive for high throughput screening.

Advantages:

- 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 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 dont have to be split saving plates and time

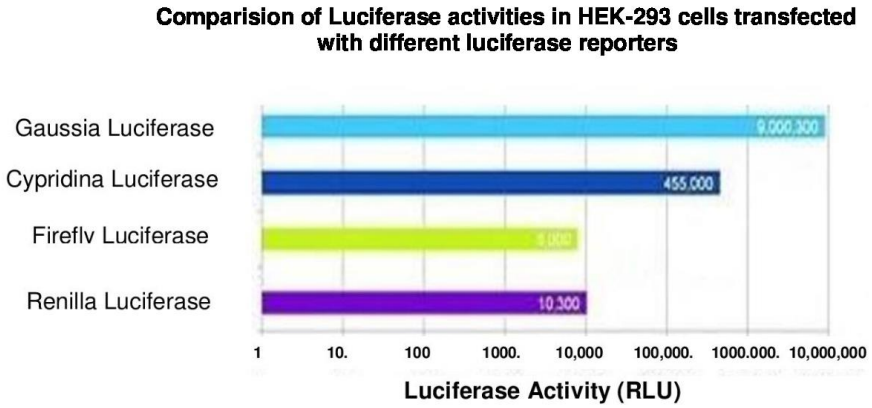

Figure 1: Comparision of luciferase reporter activities in HEK-293 cells:
HEK-293 cells were grown in reduced serum media (DMEM with 3% serum or OptiMEM1 with 0.3% serum) and transfected with plasmids expressing the indicated luciferase reporters (Gaussia, Cypridina, Firefly or Renilla) under cotnrol fo the CMV promoter using the Targefect-F1 reagent Targeting Systems) asw 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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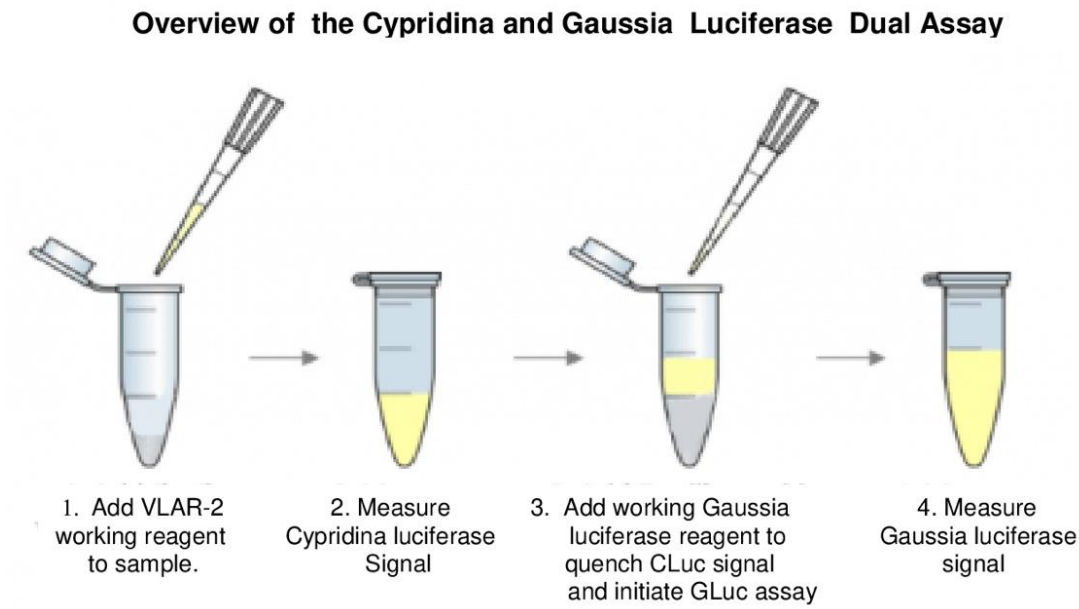

Figure 2: Overview of the Cypridina-Gaussia luciferase *Quench & Glo* ™ protocol. The assay can be performed in cell lysates supernatants or cell lysates (prepared using the cell lysis reagent (5XCLR-1) from Targeting Systems. This cell lysis reagent is designed for compatibility with the GLuc and CLuc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

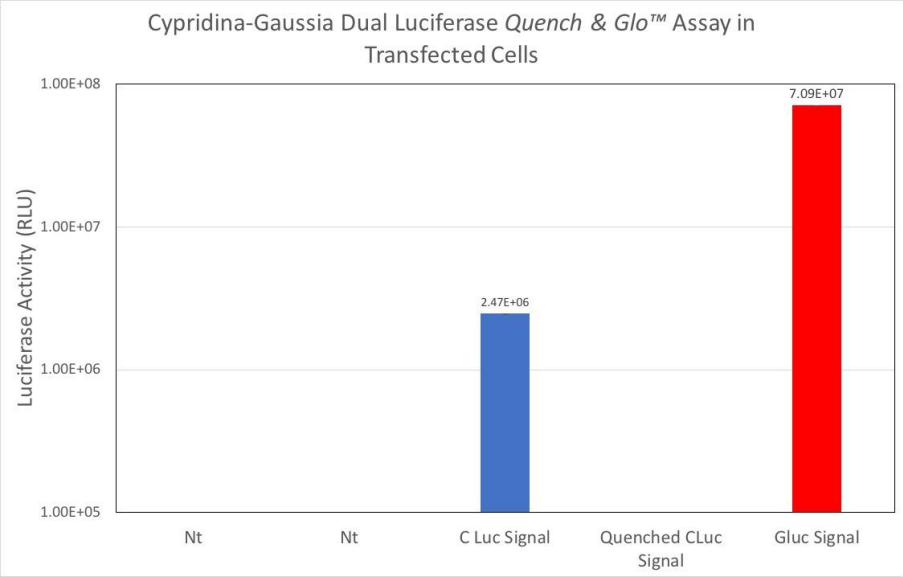

Figure 3: Sequential detection of Cypridina luciferase and Gaussia luciferase in cell supernatants using the *UltraBrite* ™ Cypridina-Gaussia dual luciferase assay reagent: Aliquots of supernatants (10 ul) from transfected HEK 293 cells were assayed with 50µL of VLAR2 reagent to measure CLuc activity. 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 & Glow reagent (RLU were 200 and 400 respectively). The quenched GLuc signal was 12000 RLU. Data shows mean of 5 determinations along with the standard deviations

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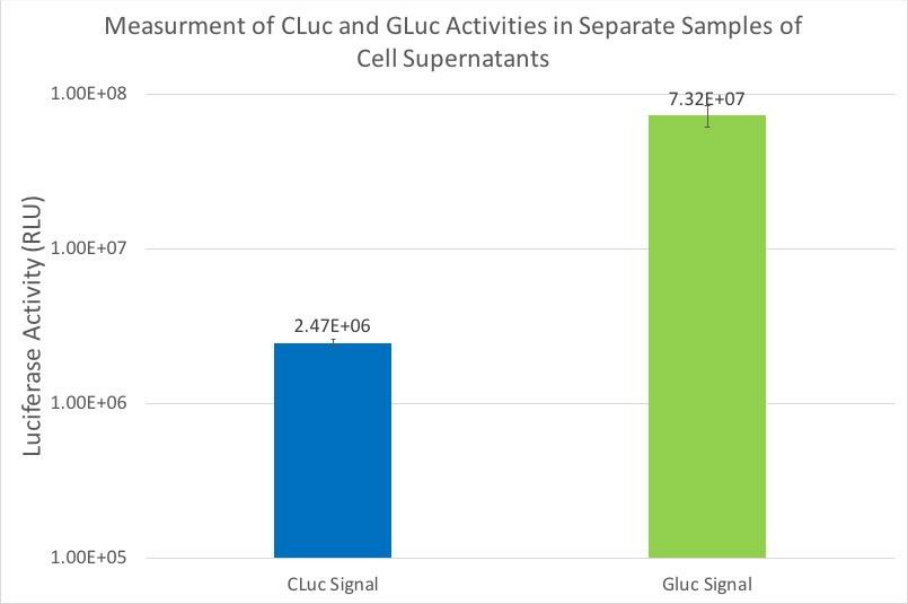

Figure 4 Measurement of Cypridina and Gaussia luciferase activities in separate samples of cell supernatnats:. Aliquots of supernatants (10 ul) from transfected HEK 293 cells were assayed with either 50µL of VLAR2 reagent to measure CLuc activity or with 50 ul of VLAR-2 buffer (no CLuc substrate) plus 50 ul of GAR Quench and Glo reagent to measure Gluc acitivty . . Data shows mean of 5 determinations along with the standard deviations

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**

Prepare working reagents by diluting 100X substrates with the respective dilution buffers

RAPID PROTOCOL:

1. Pipette 5-10 ul of cell supernatant into luminometer tueb or microplate wells
2. Add 50 ul of working VLAR-2 reagent (Component 1) of the ***UltraBrite™*** **Cypridina-Gaussia** assay reagent
3. Mix well and read CLuc activity
4. Wait 2-5 min
5. Add 50 ul of of working GAR ***Quench and Glo™*** reagent (Component 2) of the ***UltraBrite™*** **Cypridina-Gaussia** assay reagent
6. Mix well and read GLuc activity

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