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UltraBrite ™ Cypridina-Gaussia dual luciferase assay reagent

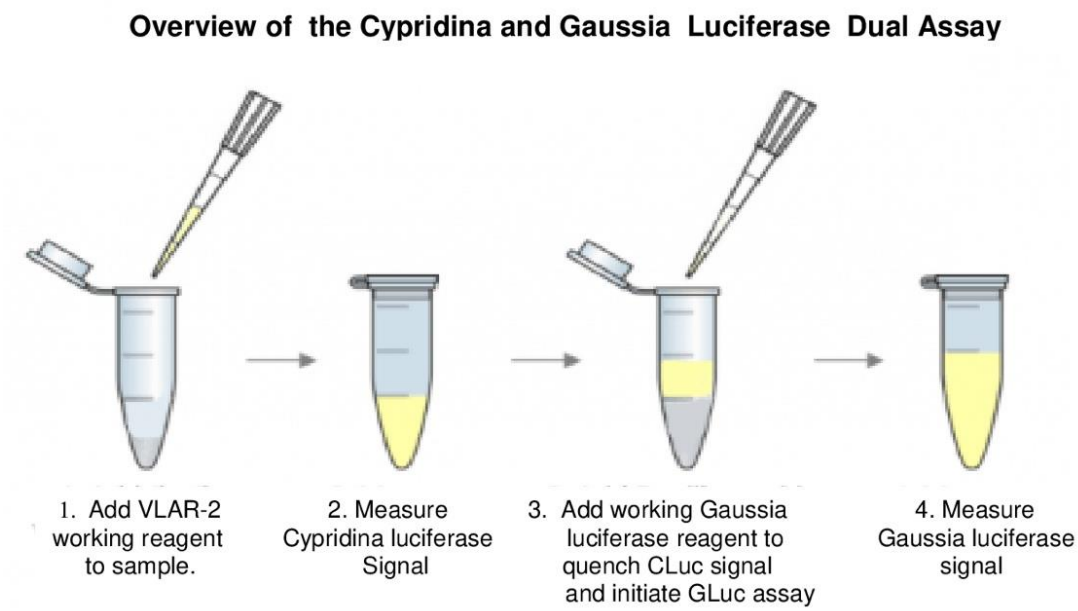

Figure 2: Overview of the Cypridina-Gaussia luciferase *Quench & Glo* ™ protocol. The assay can be performed in cell lysates supernatants or cell lysates (prepared using the cell lysis reagent (5XCLR-1) from Targeting Systems. This cell lysis reagent is designed for compatibility with the GLuc and CLuc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

COMPONENTS AND ASSAY PROTOCOL:

Component 1

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

Component 2

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

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

ASSAY PROTOCOL:

1. Pipette 5-10µl of cell 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)

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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

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

CELL LYSIS PROTOCOL:

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.
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.

TROUBLESHOOTING:
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.

Store samples at -20 ºC or -80 ºC if you wish to assay later. Luciferase activities are preserved for over a year if stored at -80 ºC.

TIME TAKEN: It takes about 15 mins to assay a 96-well plate

ANTICIPATED RESULTS:

Expect at least 99.5% quenching of CLuc activity

Expect robust acitivties of both Gluc and CLuc reporters. We usually see at least a 20X improvement in assay sensitivity compared to the Firefly /renilla dual luciferase assay (data not shown)