Lumino™ Firefly Luciferase Assay

(Cat. # 786-1267, 786-1268)

INTRODUCTION

Firefly Luciferase Assays are one of the widely used reporter assays to detect and measure gene expression, function and regulation. Firefly luciferase is widely used as reporter as it is active immediately after translation and does not require any post-translational modifications. In addition, firefly luciferase assay is highly sensitive bioluminescent assay (Fig. 1) with 100 to 1000 fold higher sensitivity when compared to CAT assay. The assay is rapid as takes only few seconds to get results.

Lumino™ Firefly Luciferase Assay is designed for rapid and high sensitive detection or quantification of firefly luciferase reporter enzyme activity from cultured bacterial or mammalian cells. Lumino™ Firefly Luciferase Assay is a flash-type luminescence assay with the signal half life of around 12 minutes (Fig. 2). The Lumino™ Firefly Luciferase Assay is highly sensitive with detection limit of 10 pg luciferase on TR717™ Microplate Luminometer. The detection limit and sensitivity range may vary depending upon luminometers. Some luminometers can detect as low as 5 fg of luciferase. This assay is rapid as including cell lysis, the assay can be done in less than 10 minutes and the assay itself take few seconds to get results.

Fig 1: Bioluminescent reaction carried out by firefly luciferase

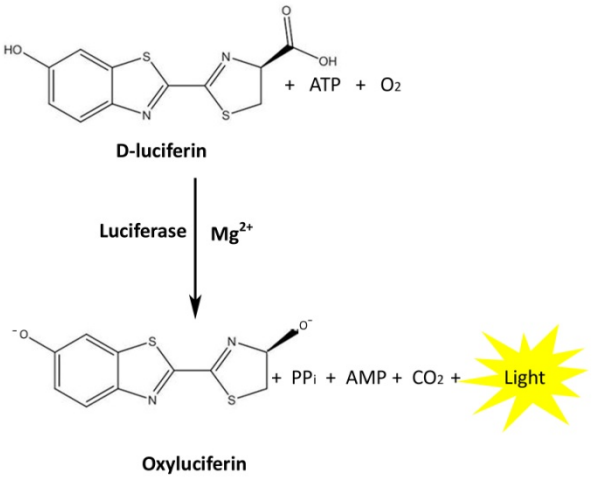
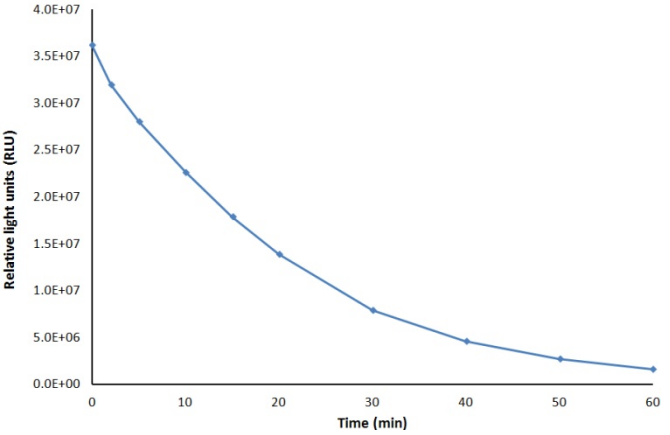


Fig 2: Lumino™ Firefly Luciferase Assay with flash kinetics



ITEM(S) SUPPLIED

Description	Cat. # 786-1267 100 assays	Cat. # 786-1268 1000 assays
Firefly Luciferase Assay Substrate	1 vial	1 vial
Firefly Luciferase Assay Buffer	10 ml	100 ml
Luciferase Cell Lysis Buffer [5X]	10 ml	100 ml

STORAGE CONDITIONS

The kit is shipped on blue ice. Store the kit at -20°C. Luciferase Cell Lysis Buffer [5X] can be stored at 4°C. Working Assay Solution formed by mixing of Firefly Luciferase Assay Substrate and Firefly Luciferase Assay Buffer is stable for 2 months at -20°C in dark. Store the Working Assay Solution in aliquots at -70°C in dark for long term storage. When stored as directed, the kit is stable for 1 year.

IMPORTANT INFORMATION

- Bring the kit components to room temperature before performing assay.
- Avoid exposing reagents to excessive heat or light as they can get degraded.
- After cell lysis, perform the assay immediately or store on ice for short term storage. For long term storage or assaying later store the cell lysate at -70°C.
- Assay reagents may contain corrosive chemical. Always wear gloves before performing the assay to avoid any contact with reagents or treated samples. It is advised to check the SDS of the assay kit and the drugs or chemicals used to treat cells before performing assay.

ADDITIONAL ITEMS REQUIRED

- PBS (Cat. # 786-377)
- Lysozyme (Cat. # 786-037) for bacterial cell lysis
- Luciferase (Cat. # 786-1308). This is optional for standard plot or positive control
- Luminometer
- White 96-well micro titer plates or luminometer tubes depending upon the luminometer type.

PREPARATION BEFORE USE

1. Prepare 1 X Luciferase Cell Lysis Buffer by diluting Luciferase Cell Lysis Buffer [5X] with deionized water in ratio 1:4.
   NOTE: *The Luciferase Cell Lysis Buffer is sufficient 100 wells of 6-well plate cultures. For more please order extra bottle*
2. For bacterial cell lysis, add 150,000 units or 1.25 mg of lysozyme per 1 ml of 1 X Luciferase Cell Lysis Buffer
3. Add the entire contents of the Firefly Luciferase Assay Buffer bottle to the Firefly Luciferase Assay Substrate and dissolve the substrate in the assay buffer with the help of a pipette to make Working Assay Solution. Make small one time use aliquots protected from light. Store the unused vials at -70°C in dark.

PROTOCOL

Preparation of Cell Lysates

1. Aspirate or remove the cell culture medium.
   NOTE: *The cells should not be over-confluent, preferably form only a monolayer*
2. Wash the cells with pre-warmed PBS.
3. Add 1 X Luciferase Cell Lysis Buffer enough just to cover the cells. Check the table below

Wells per plate	Luciferase Cell Lysis Buffer per well
6	500 µl
12	250 µl
24	100 µl
48	65 µl
96	20 µl

4. Rock the culture plates on rocker or shaker for 1 minute and then scarpe the cells with a cell scraper.
5. Transfer cell lysate to a microfuge tube and vortex for 10 seconds.
6. Incubate the tube at room temperature for 5 minutes to perform cell lysis.
7. Vortex for 10 seconds and centrifuge at 12,000 g for 30 sec at 4°C.
8. Collect the supernatant. Use it immediately for assay or store at -70°C.

Preparation of Bacterial Cell Lysates

1. Centrifuge 1ml of bacterial culture (approx. OD_{600nm} = 0.6) for 3 minutes at 5000 g at 4°C.
2. Perform a freeze thaw by freezing the pellet on dry ice or -20°C and then allow to warm at room temperature.
3. Add 0.5 ml of 1 X Luciferase Cell Lysis Buffer containing lysozyme to the bacterial cell pellet and vortex.
4. Incubate for 10 minutes at room temperature.
5. Pellet the cells at 12,000 g for 1 minute at 4°C.
6. Collect the supernatant. Use immediately for the assay or store at -70°C.

Standard Luciferase Aassy Protocol for Manual Luminometer

1. Switch on the luminometer.
2. Set the software of luminometer to perform 2-second measurement delay or minimum indicated by the software followed by 10-second luminescence measurement per sample or well.
3. Add 100 µl of Working Assay Solution (*Check section: Preparation Before Use*) to each well of 96-well microtiter white plate or luminometer tube.
4. Add 20 µl of the cell lysate (containing firefly luciferase) per well or tube and mix gently with a pipette.
5. Place the plate or tube in the luminometer and start the program.
6. Record or save the firefly luciferase activity readings.

NOTE: *For luminometer equipped with automatic injectors, add 20 µl lysate per well or tube and adjust the automatic setting to dispense 100 µl of working Assay Solution as per manufacturer's instruction.*
NOTE: *Lumino™ Firefly Luciferase Assay is compatible with measurement using scintillation counters.*

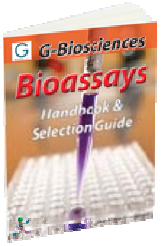
TROUBLESHOOTING

Issue	Suggested reason	Possible solution
No or low luminescence	Low transfection efficiency	Optimize transfection conditions, check quality of DNA, use actively dividing cells or cells with low passage number or change the cell line
	No promoter activity	Optimize promoter activation conditions or incubate cells longer or change the growth conditions to optimize expression
	Reagents degraded as not stored	Take another vial -70°C

	properly	stored vial of Working Assay Solution. Store reagents as directed. Order new set if the reagents were not stored as directed.
	Low luciferase expression	Increase intergration time of instrument or scale-up the volume of sample and reagent. Alternatively lyse cell is smaller volume of 1 X Luciferase Cell Lysis Buffer
	Luciferase got degraded	Store cell lysates on ice or use fresh batch of cells and lyse and assay immediately
	Luminometer not adjusted as per its sensitivity	Increase intergration time of instrument or scale-up sample volume
High luminescence	High luciferase expression or luminometer settings not optimized	Decrease the integration time of the luminometer or dilute the sample
High background luminescence	Control cells/sample were contaminated	Incubate cells for longer time or use fresh batch of control cells/sample

RELATED PRODUCTS

Download our Bioassays Handbook.



http://info2.gbiosciences.com/complete-bioassay-handbook

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