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A Geno Technology, Inc. (USA) brand name

Lumino™ Firefly Luciferase Glow Assay

(Cat. # 786-1353, 786-1354, 786-1359)



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INTRODUCTION 3

ITEM(S) SUPPLIED 4

STORAGE CONDITIONS 4

SPECIFICATIONS 4

IMPORTANT INFORMATION 5

ADDITIONAL ITEMS REQUIRED 5

PREPARATION BEFORE USE 5

PROTOCOL 5

TROUBLESHOOTING 6

RELATED PRODUCTS 7

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 Glow Assay is designed for high throughput screening or quantification of luciferase expression in mammalian cells which is mainly performed by batch processing of 96- and 384- well plates. Lumino™ Firefly Luciferase Glow Assay is homogenous, high-sensitive, glow type (long-lived) bioluminescent assay with signal half-life of more than 3 hrs (Fig. 2). A long signal half-life (>3hrs) of the luminescent assay is necessary for batch-processing (HTS) systems, where a large number of plates can be stacked after assay and before measurement.

Fig 1: Bioluminescent reaction carried out by firefly luciferase

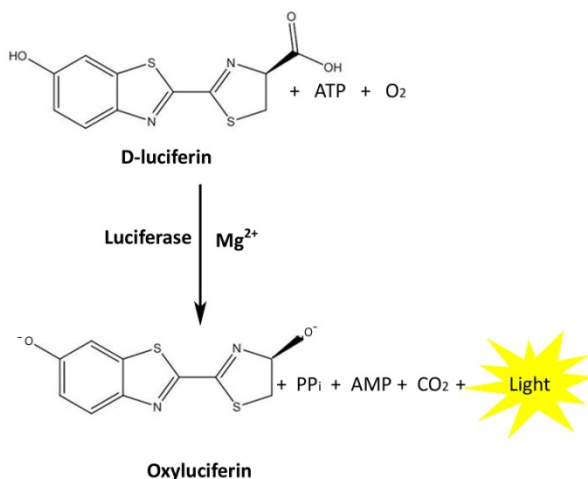
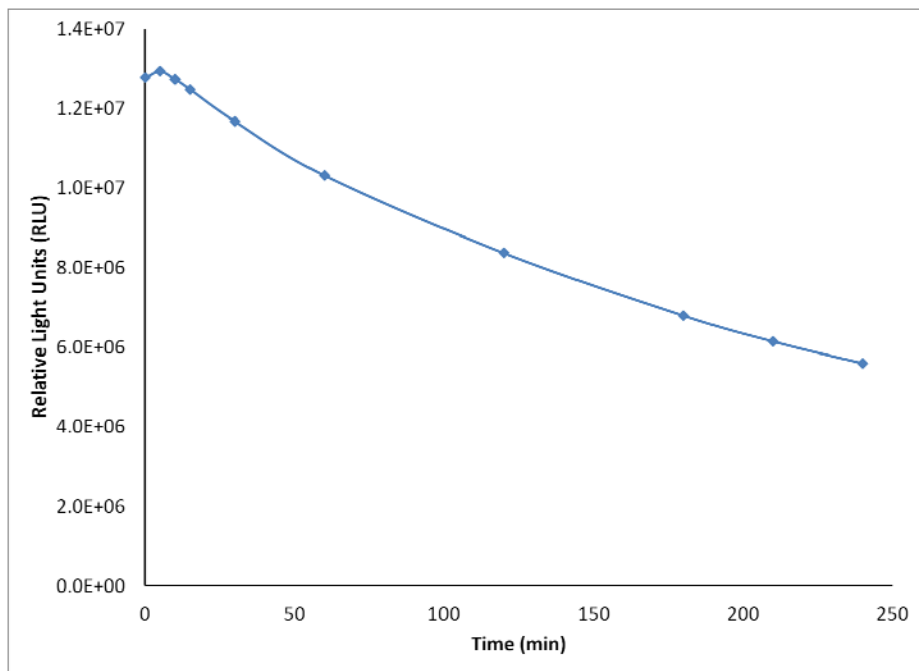


Fig 2: Lumino™ Firefly Luciferase Glow Assay with Glow kinetics



ITEM(S) SUPPLIED

Description	Cat. # 786-1353 100 assays	Cat. # 786-1354 500 assays	Cat. # 786-1359 1000 assays
Firefly Luciferase Glow Assay Substrate	1 vial	1 vial	2 vials
Firefly Luciferase Glow Assay Buffer	10 ml	50 ml	2x 50 ml

STORAGE CONDITIONS

The kit is shipped on blue ice. Store the kit at -20°C. Firefly Luciferase Glow Assay Buffer can be stored at 4°C. Firefly Luciferase Glow Assay Substrate after reconstitution with Firefly Luciferase Glow Assay Buffer should be stored in small aliquots protected from light at -70°C.

SPECIFICATIONS

- **High sensitivity:** High sensitive detection of firefly luciferase activity.

- **Suitable for HTS assays:** Designed for batch-processing of mammalian cells. Single reagent is added for cell lysis and luminescent assay which makes HTS assays faster to perform.
- **Stable signal:** Glow type bioluminescent assay with signal half-life of greater than 3 hrs.
- **Excellent linearity:** Linear over high-log order (5 or more) of luciferase concentration. The linear range is also dependent of the sensitivity of the luminometer.
- Assay reagents compatible with other firefly luciferases.

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.
- Working Assay Solution formed by mixing of Firefly Luciferase Glow Assay Substrate and Firefly Luciferase Assay Glow Buffer is stable for 2 months at -20°C in dark. Store the working solution in aliquots at -70°C in dark for long term storage.

ADDITIONAL ITEMS REQUIRED

- Reagents and instruments for culturing, propagating and treating mammalian cell cultures.
- PBS (G-Biosciences, Cat. # 786-377)
- Luminometer or other luminescence monitoring instrument
- White or black opaque 96-well or 384-well micro titer plates.

PREPARATION BEFORE USE

Add entire contents of one bottle of Firefly Luciferase Glow Assay Buffer to one vial of Firefly Luciferase Glow Assay Substrate and dissolve the substrate in buffer to make Working Assay Solution. Make small one time use aliquots of Working Assay Solution in brown bottles or protected from light. Keep the ones for immediate use and store rest at -70°C.

PROTOCOL

Firefly luciferase Glow Assay

1. 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.
2. Bring the mammalian cells to be tested for luciferase to room temperature.
3. Bring the PBS used for washing the cells to room temperature.
4. Wash the cells once with PBS.
5. Add 100 µl Working Assay Solution per well in 96-well plate and place the plate on shaker for 10 minutes in dark to perform cell lysis.

NOTE: The plates must be compatible with the luminometer being used.

6. Place the plate in the luminometer and measure the luminescence.

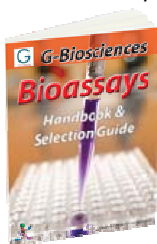
NOTE: Lumino™ Firefly Luciferase Assay is compatible with measurement using Scintillation Counters.

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 properly	Take another vial -70°C 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.
	Luminescence reading not taken within 1-2 hrs of assay	The luminescent signal half-life is 3 hrs, so its recommended to assay within 2 hrs
	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>

For other related products, visit our website at www.GBiosciences.com or contact us.



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