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Firefly Luciferase Assay Kit

Product Information

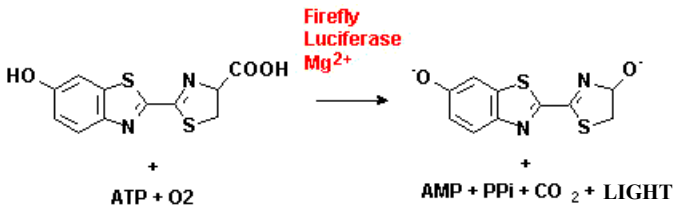
Name :	***Firefly* Luciferase Assay Kit**		
Catalog Numbers :	FP-BE794A 150 tests		
	FP-BE794B 1000 tests		
Product Components :		150 tests	1000 tests
	D-Luciferin	3 vials (1 mg)	2 vials (10 mg)
	Firefly Luciferase Assay Lysis Buffer 5X	15 ml	2 x 15 ml
	Firefly Luciferase Assay Buffer	15 ml	100 ml

Storage: -80°C (Q) or -20°C (short term) Protect from light and moisture
Store the kit at -20°C or below. Firefly Luciferase Assay Buffer is stable at -20°C for three months and at -80°C for at least six months from date of receipt. The other kit components are stable at -20°C for at least six months from date of receipt. Kit components and D-luciferin stock solutions in water are stable to at least 5 freeze-thaw cycles.
Note: Sufficient firefly lysis buffer is provided to perform the stated number of assays with cells grown in 96 – 24 well plates. For applications requiring more lysis buffer (e.g. >100 uL/well), additional 5X lysis buffer (Cat. # FP-BE7941) may be purchased separately.

Introduction

Firefly luciferase is widely used as a reporter for studying gene regulation and function, and for pharmaceutical screening. [1,2] It is a very sensitive genetic reporter due to the absence of endogenous luciferase activity in mammalian cells or tissues. [3,4] Firefly luciferase is a 62,000 Dalton protein, which is active as a monomer and does not require subsequent processing for its activity. The enzyme catalyzes ATP-dependent D-luciferin oxidation to oxyluciferin producing light emission centered at 560 nm (Figure 1). Firefly luciferase follows Michaelis-Menten

kinetics and, as a result, maximum light output is not achieved until the substrate and co-factors are present in large excess. When assayed under these conditions, light emitted from the reaction is directly proportional to the number of luciferase enzyme molecules. This firefly luciferase assay kit is designed for simple and efficient



quantitation of firefly luciferase reporter enzyme activity from cultured cells with high sensitivity and linearity (Figure 2).

Bioluminescent reaction catalyzed by *Firefly* luciferase.

This is a flash-type luminescence assay that requires signal to be measured immediately after adding working solution to samples. The luminescence signal decreases about 50% after about 10 minutes of reaction time (Figure 3), although signal half-life may vary depending on luciferase expression levels. FluoProbes also offers the Steady-Luc HTS Firefly Assay Kit (cat. no. FP-BU6870), which is a homogenous glow-type assay with signal half-life of 3-5 hours (see related products).

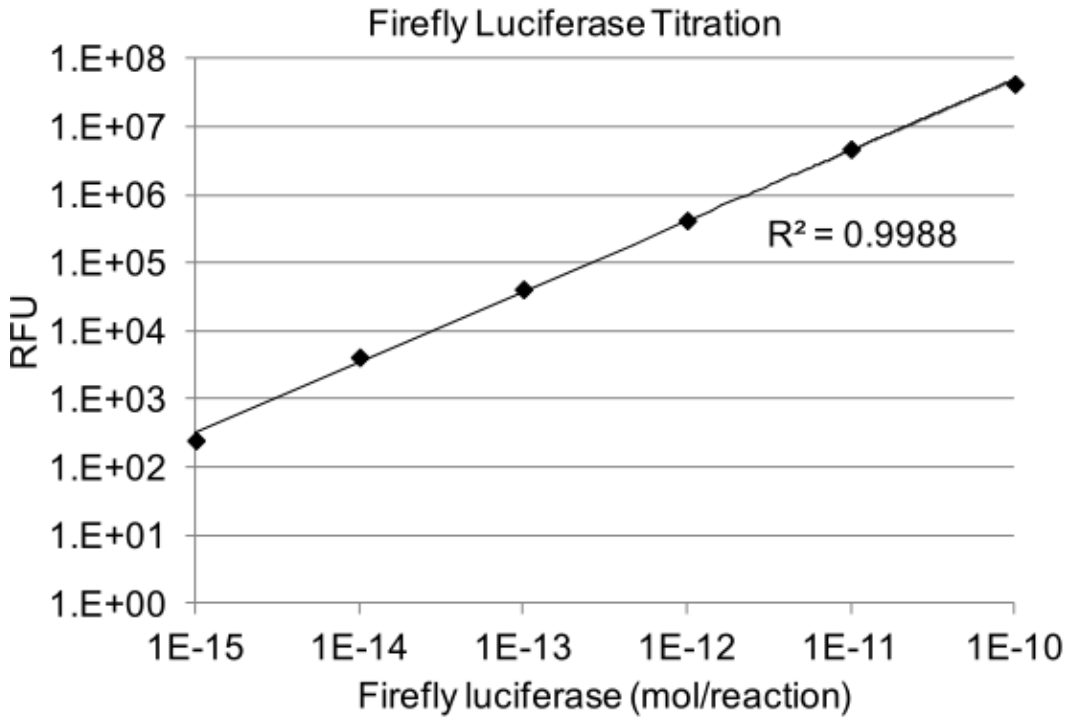


Figure 2. Titration of recombinant firefly luciferase in the Firefly Luciferase Assay. Quantilum® Luciferase (Promega) was serially diluted in 1X Firefly Lysis Buffer with 1 mg/mL BSA and measured in the assay. Luminescence was measured on a Promega Glomax® 20/20 single tube luminometer with integration time of 1 second. Background from reagents without enzyme added was subtracted from luminescence values.

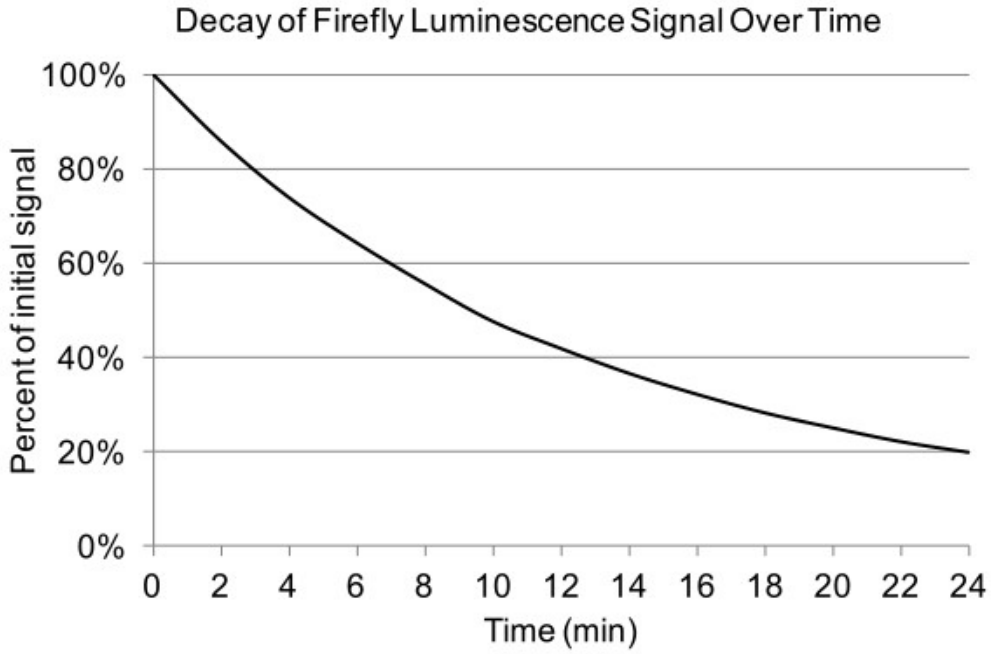


Figure 3. Stability of luminescence signals in the Firefly Luciferase assay. Luminescence measurements were carried out in a white 96-well plate on cells transfected with firefly luciferase. Luminescence was measured using a Bio-Tek H1m microplate reader every 2 minutes for 24 minutes, and RLU values were normalized to the first measurement for each reaction.

Directions for use

Protocol - Preparation of Cell Lysates

A. Preparation of *Firefly* Luciferase Lysis Buffer

Firefly Luciferase Lysis Buffer 1X working solution is prepared by adding 1 volume of 5X *Firefly* Luciferase Lysis Buffer to 4 volumes of distilled water and mixing well. The 1X Lysis Buffer may be stored at 4°C for up to one month. Store the 5X *Firefly* Luciferase Assay Lysis Buffer at -20°C.

B. Lysis of Cells Cultured in Multiwell Plates

1- Remove the growth medium from the cultured cells and gently wash the cells once with a sufficient volume of phosphate buffered saline (PBS) to cover the surface of the culture vessel. Remove the PBS and add 1X passive lysis buffer using the volume recommended below for each type of well:

6 well culture plate 500 µl
12 well culture plate 250 µl
24 well culture plate 100 µl
48 well culture plate 65 µl
96 well culture plate 20 µl

2- Place the culture plates on a rocking platform or orbital shaker with gentle rocking/shaking to ensure complete and even coverage of the cell monolayer with 1X *Firefly* Luciferase Lysis Buffer. Rock the culture plates at room temperature for 15 minutes.

***Note**: Cultures that are overgrown are often more resistant to complete lysis and typically require an increased volume of firefly luciferase lysis buffer and/or an extended treatment period to ensure complete lysis. Lifting cells from the plate will facilitate the process of cell lysis. Mini cell scrapers (cat. no. IWV240) are available for harvesting lysates from 96-, 24-, and 48-well plates*

3- Transfer the lysate to a tube or vial. Optional: the lysate can be cleared by centrifugation for 30 seconds at top speed in a refrigerated microcentrifuge and transferred into a new tube. Place at 4°C for until ready to assay. Store lysates at -20°C or -80°C if assay will not be performed on the same day.

Protocol - *Firefly* Luciferase Assay

A. Preparation of *Firefly* Luciferase Assay Solution

1- Thaw a bottle of *Firefly* Luciferase Assay Buffer at room temperature.
2- Prepare 10 mg/mL D-luciferin stock solution. For component FP-BE794d (1 mg), add 100 uL water to the vial and mix. For component FP-BE794c (10 mg), add 1 mL water to the vial and mix. The stock solution can be stored for at least 6 months at -20°C or below, and is stable to up to 5 freeze/thaw cycles.
3- Prepare enough firefly working solution to perform the desired number of assays (100 uL working solution per assay). Add D-luciferin (10 mg/mL) to assay buffer at a ratio of 1:50. For example, add 20 uL D-luciferin stock solution to 1 mL firefly assay buffer.

Note: For best results, working solutions (assay buffer with substrate) should be prepared fresh before each use, and used within 3 hours of preparation. Firefly working solution activity decreases ~10% after 3 hours and ~25% after 5 hours at room temperature.

B. Standard Protocol

For manual luminometer:

1- Set up luminometer with appropriate parameters (delay time, integration time, sensitivity, etc.). We routinely use integration time of 1 second.

2- Add 20 µl of cell lysate into a reaction tube that is compatible with your luminometer.

3- Add 100 uL of firefly working solution to the reaction tube and mix by pipetting or vortexing.

4- Immediately place tube in luminometer and record the firefly luminescence measurement.

For luminometer with injector:

1- Format the luminometer so that the injector dispenses 100µl. Prime the injector with *Firefly* Luciferase Assay Solution.

2- For each reaction, carefully add 20µl of cell lysate to an individual luminometer tube or to the wells of a multiwell plate.

3- Place the samples in a luminometer.

4- Initiate measurement. This action will cause *Firefly* Luciferase Assay Solution to be injected into the reaction vessel and the measurement to be subsequently taken. Luminescence is normally integrated over 10 seconds without delay. Other integration times may also be used.

5- Record the *Firefly* luciferase activity measurement.

6- If using a single tube luminometer, discard the reaction tube, and proceed to the next *Firefly* Luciferase Assay reaction. If using a plate luminometer, the luminometer will automatically begin injecting *Firefly* Luciferase Assay Solution into the next well indicated on the luminometer plate.

Determination of Assay Background

The expression of a luciferase reporter is quantified by the luminescence produced above background levels. In most cases, background created by the reagent in the absence of luciferase is very low compared to signal with luciferase. However, when measuring low levels of luciferase activity, it is important to subtract the background signal from untransfected cells or cells transfected with a negative control vector from measurements of luciferase activity.

References

1- Alam, J., *et al.*, "Reporter genes: Application to the study of mammalian gene transcription.", *Anal. Biochem.*, **188**, 245 (1990)
2- Bronstein I., *et al*., "Chemiluminescent and bioluminescent reporter gene assays.", *Anal. Biochem*., **219**, 169 (1994).
3- Gould S.J., *et al.*, "*Firefly* luciferase as a tool in molecular and cell biology.", *Anal. Biochem*., **175**, 5 (1988)
4- Brasier, A.R., *et al*., "Optimized use of the *Firefly* luciferase assay as a reporter gene in mammalian cell lines", *BioTechniques*., **7**, 1116 (1989)

Related products

- 5X Firefly Lysis Buffer, FP-BE7941
- Mini Cell Scrapers, IWV240
- Firefly Luciferase Assay Kit (Lyophilized), FP-1F8170
- *Renilla* Luciferase Assay Kit, BE7932
- *Firefly* & *Renilla* Luciferase Assay Kit, FP-BE7812
- *Firefly HTS* Luciferase Assay Kit, FP-BU6870
- Growth plate 96x1ml, sterile BS6200
- Growth plate 96x2ml, sterile BS6210

Ordering information

Catalog size quantities and prices may be found at http://www.fluoprobes.com
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06

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