

Firefly Luciferase HTS Assay Kit

Product Information

Name :	Firefly Luciferase Assay Kit for 96, 384 and 1536-well plates (3-5 hours reading)		
Catalog Numbers:	FP-BU6870	10 ml (100, 400 or 3300 tests in 96, 384 or 1536-well plate)	
	FP-BU6871	100 ml (1000, 4000 or 33000 tests in 96, 384 or 1536-well plate)	
Components:		10 ml	100 ml
	D-Luciferin,	2.5 mg	25 mg
	Firefly Luciferase Assay Buffer	10 ml	100 ml

Storage: -70°C (Q) or -20°C (short term) Protect from light and moisture
 The components of the kit are stable at -70°C for >6 months (3 months at -20°C). *Firefly* luciferase assay solution (Assay Buffer + Substrate) should be prepared fresh for each use. Avoid repeated freeze-thaw cycles. Aliquot *Firefly* Luciferase Assay Buffer for storage if necessary.

Introduction

Firefly luciferase is widely used as a reporter gene for studying gene regulation and function, and for pharmaceutical screening. It is a very sensitive genetic reporter due to the lack of any endogenous activity in mammalian cells or tissues. The *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 by oxygen into oxyluciferin with emission of light centered on 560 nm (figure 1).

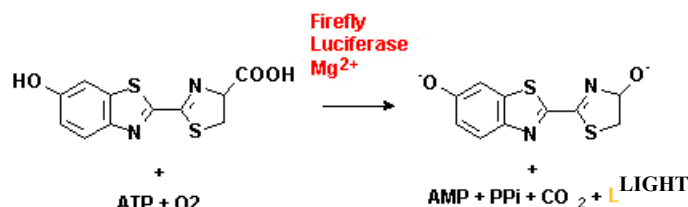
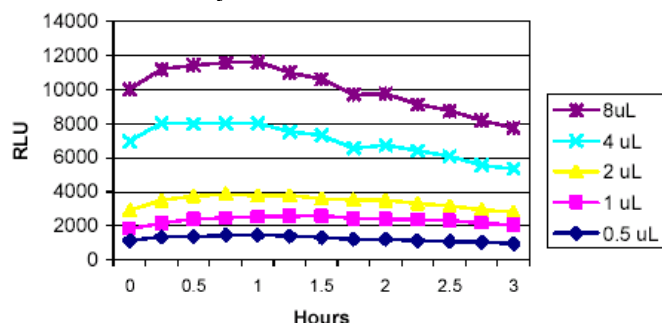


Figure 1: Bioluminescent reaction catalyzed by *Firefly* luciferase.

However, the light production resulting from the reaction leads to formation of suicidal adenyloxyluciferin at the enzyme surface. It results in very short half-life of the light emission with a flash-type kinetics. Several substances have been described to prolong light production by regenerating enzyme through removing inhibitory oxyluciferin from the enzyme surface. But the duration (10-15 min) is still too short for batch process screening.

FluoProbes' Luciferase HTS assay system is a proprietary mixture of substances that modify the enzymatic reaction to produce a long lasting signal (steady glow) by preventing the formation of adenyloxyluciferin at the enzyme surface. It is a homogeneous high sensitivity firefly luciferase reporter gene assay kit with a half-life of 3-5 hours for the quantification of firefly luciferase expression in mammalian cells. This kit is specially designed for batch processing systems using high-density microplates such as 384- and 1536-well plates, in high throughput environments. In addition, this system offers higher sensitivity and wider dynamic range for detecting luciferase activity within mammalian cells compared to similar systems offered by other vendors (figure 2).

FluoProbes' Steady Glow Kinetic Plot



Company A's Steady Glow Kinetic Plot

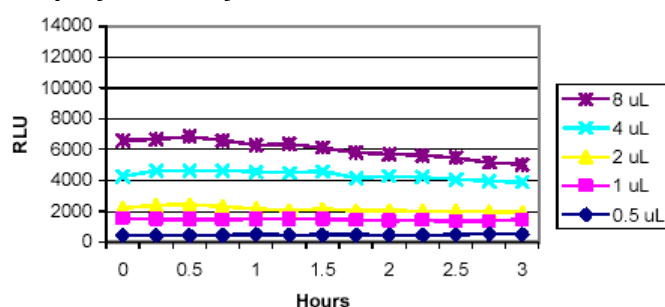
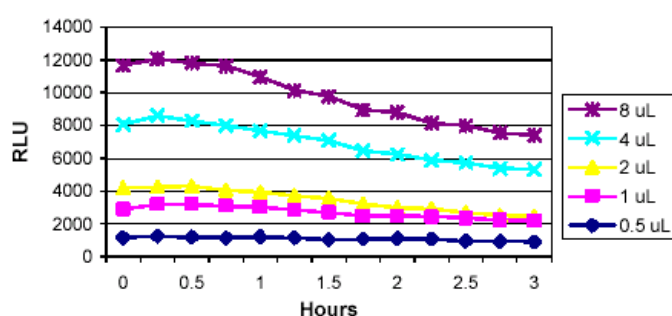


Figure 2: Comparison of Steady Glow Kinetics between FluoProbes' HTS kit and competitors' kits.

80% confluent PC3 cells in each 96 well were transfected with 0.5, 1, 2, 4 or 8 uL transfection mixture containing firefly luciferase plasmid and Fugene 6 (Roche) respectively. 24 hours after transfection, 100 µL of *Firefly* Assay Solution from our HTS kit or similar solutions from competitors' kits were added into corresponding wells. After 5 minutes to allow completion of cell lysis, the 96-well plate was then placed in a MicroLumi 96 Microplate Luminometer (Harta Instruments) for luminescence measurement. RLU: Relative Luminescence Units.

Company B's Steady Glow Kinetic Plot



Directions for use

Protocol

- 1- Equilibrate the kit components to room temperature (22 °C) before reconstitution.
- 2- To prepare HTS *Firefly* Assay Solution, reconstitute one vial of lyophilized substrate with 10 mL or 100 mL of HTS *Firefly* Assay Buffer. Mix well the contents of the vial by inversion until the substrate is completely dissolved. Only prepare reagents as needed for one day.
- 3- Remove 96- or 384-well plates containing mammalian cells from the incubator. The plates must be compatible with the luminometer used for luminescence measurements.
- 4- Add the amount of the reagent equal to that of the culture medium in each well and mix. For 96-well plates: add 100 µL to each well containing 100 µL of cells in medium. For 384-well plates: add 25 µL to each well containing 25 µL of cells in medium. For 1536-well plates: add 3 µL to each well containing 3 µL of cells in medium.
- 5- Wait at least 5 minutes for complete lysis of the cells, then measure luminescence with a microplate luminometer.

References

- Alam, J., et al., "Reporter genes: Application to the study of mammalian gene transcription.", *Anal. Biochem.*, **188**, 245 (1990)
- Bronstein I., et al., "Chemiluminescent and bioluminescent reporter gene assays.", *Anal. Biochem.*, **219**, 169 (1994).
- Gould S.J., et al., "Firefly luciferase as a tool in molecular and cell biology.", *Anal. Biochem.*, **175**, 5 (1988)
- Brasier, A.R., et al., "Optimized use of the *Firefly* luciferase assay as a reporter gene in mammalian cell lines", *BioTechniques.*, **7**, 1116 (1989)
- Wood K., *Recent advantages and prospects for use of beetle luciferases as genetic reporters*. In: Bioluminescence and Chemiluminescence current status. Proceedings of the Vth International Symposium on Bioluminescence and Chemiluminescence, Cambridge, Ed. by P.Stanley and L. Kricka, p543 (1990)
- Airth R. et al.: *The functioning of Coenzyme A in luminescence*. Biochemica and Biophysica Acta 27:519-532 (1958).

Related / associated products and documents

See [BioSciences Innovations catalogue](#) and [e-search tool](#).

- *Renilla* Luciferase Assay Kit, [FP-BE7930](#)
- *Firefly* Luciferase Assay Kit, [FP-BE7940](#)
- *Firefly & Renilla* Luciferase Assay Kit, [FP-BE7810](#)
- 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

Disclaimer : Materials from FluoProbes® are sold **for research use only**, and are not intended for food, drug, household, or cosmetic use. FluoProbes® is not liable for any damage resulting from handling or contact with this product.

Rev.G09E-F08VB