# Steady-Luc *Firefly* HTS Assay Kit

## Catalog Number: 30028-1, 30028-2 & 30028-3

Revised December 13, 2012


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## 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 lack of any endogenous activity in mammalian cells or tissues[3, 4]. 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 560nm (Figure 1).

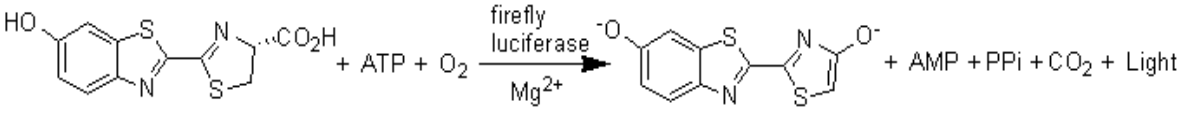
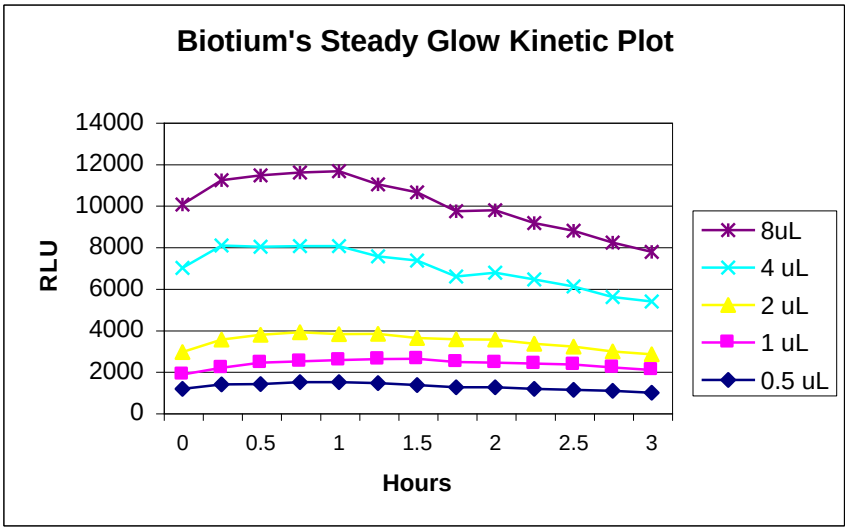



***Figure 1*. Bioluminescent reaction catalyzed by *Firefly* luciferase**

However, the light production resulting from the reaction leads to formation of suicidal adenyl-oxyluciferin 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[5, 6]. But the duration (10-15 min) is still too short for batch process screening.

Biotium's Steady-Luc 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 adenyl-oxyluciferin 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).



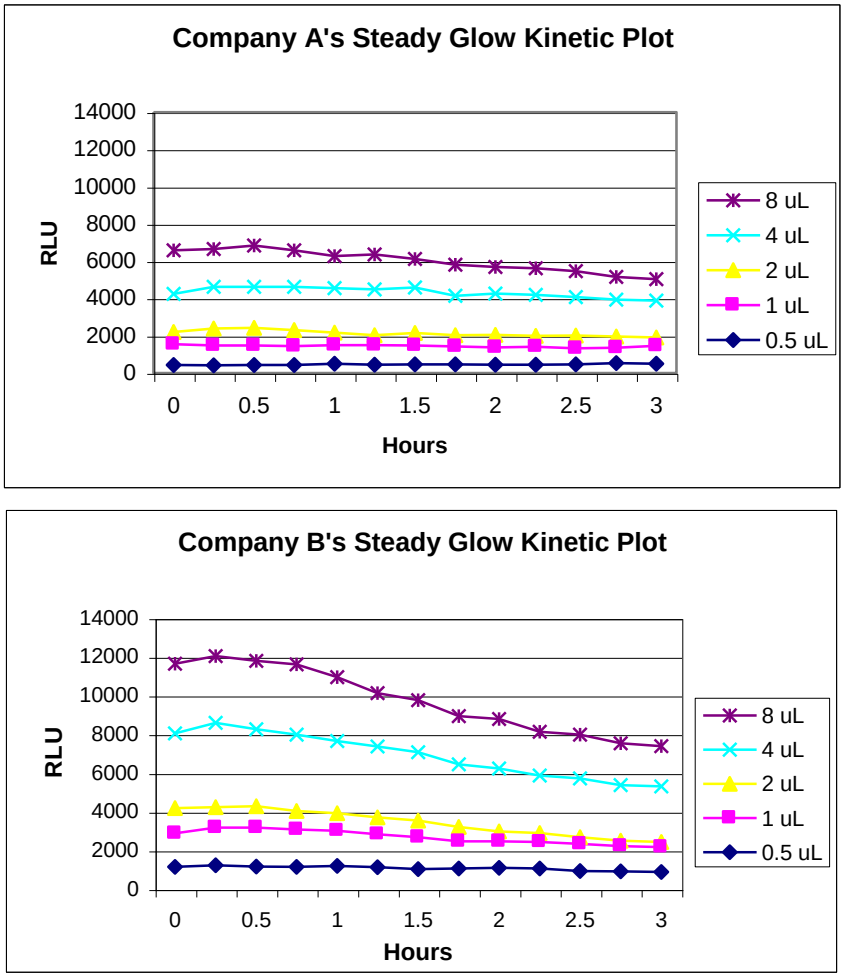



***Figure 2:*** **Comparison of Steady Glow Kinetics between Biotium's Steady-luc kit and competitors' kits.** 80% confluent PC3 cells in each 96 well were transfected with 0.5, 1, 2, 4 or 8 uL transfection mixture containing firefly luciferase plasmid and Fugene 6 (Roche) respectively. 24 hours after transfection, 100 uL of *Firefly* Steady-luc Assay Solution from Steady-luc 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 MicroLumi96 Microplate Luminometer (Harta Instruments) for luminescence measurement. RLU: Relative Luminescence Units.

## Product components

**Steady-Luc *Firefly* HTS Assay Kit, 30028-1 (100 assays)**

3 x 1 mg D-Luciferin

1 x 12 mL Steady-Luc *Firefly* Assay Buffer

***Note:*** *Using the recommended assay volumes of 100 µL for 96-well microplates, 25 µL for 384-well microplates and 3 µL for 1536-well microplates, this kit is sufficient for 100, 400 and 3,300 assays respectively.*

**Steady-Luc *Firefly* HTS Assay Kit, 30028-2 (1,000 assays)**

1 x 25 mg D-Luciferin

1 x 100 mL Steady-Luc *Firefly* Assay Buffer

***Note:*** *Using the recommended assay volumes of 100 µL for 96-well microplates, 25 µL for 384-well microplates and 3 µL for 1536-well microplates this kit is sufficient for 1,000, 4,000 and 33,000 assays respectively.*

**Steady-Luc *Firefly* HTS Assay Kit, 30028-3 (10,000 assays)**
10 x 25 mg D-Luciferin
10 x 100 mL Steady-Luc *Firefly* Assay Buffer
***Note:*** *Using the recommended assay volumes of 100 µL for 96-well microplates, 25 µL for 384-well microplates and 3 µL for 1536-well microplates this kit is sufficient for 10,000, 40,000 and 330,000 assays respectively.*

## Storage Conditions

Store Steady-Luc *Firefly* HTS Assay Kit at –70°C. Kit components are stable for six months at –70°C. Steady-Luc *Firefly* Assay Solution (Assay Buffer + Substrate) should be prepared fresh for each use. Avoid repeated freeze-thaw cycles. Aliquot Steady-Luc *Firefly* Assay Buffer for storage if necessary.

## Assay Procedure

1. Equilibrate the kit components to room temperature (22 °C) before reconstitution.

2. To prepare Steady-Luc *Firefly* Assay Solution, mix lyophilized substrate and Steady-Luc *Firefly* Assay Buffer in 1 mg to 4 mL ratio. For each 1 mg vial of lyophilized substrate, mix with 4 mL Steady-Luc *Firefly* Assay Buffer. For each 25 mg vial lyophilized substrate, mix with 100 mL Steady-Luc *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.

   ***Note:*** *D-luciferin in assay buffer has limited stability. If you need less than 4 mL or 100 mL assay solution, you may dissolve D-luciferin in DI water as 10X or 50X stock solution and store it at -20$^{o}$C or below for repeated use. The D-luciferin stock solution should be stable for at least one month, depending on the frequency of freeze-thaw cycles. The required volume of working solution can be prepared by diluting the stock solution in Steady-Luc Firefly Assay Buffer to a final concentration of 0.25 mg/mL D-luciferin.*

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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