

# Product Information

## Steady-Luc™ Firefly HTS Assay Kit

Catalog Number: 30028-T, 30028-1, 30028-2, 30028-3

### Kit Contents

Component	30028-T (40 assays)	30028-1 (120 assays)	30028-2 (1000 assays)	30028-3 (10,000 assays)
D-Luciferin	99907 1 mg	99907 3 x 1 mg	30028A2 25 mg	30028A2 10 x 25 mg
Steady-Luc Assay Buffer	30028B-T 4 mL	30028B 12 mL	30028B2 100 mL	30028B2 10 x 100 mL

Number of assays is based on 96-well plate format.

### Storage and Handling

Store Steady-Luc Firefly HTS Assay Kit at  $-70^{\circ}\text{C}$ . Kit components are stable for at least six months from date of receipt when stored as recommended. Avoid repeated freeze-thaw cycles. Aliquot Steady-Luc Firefly Assay Buffer for storage if necessary. Steady-Luc Firefly Assay Solution (Assay Buffer + Substrate) should be prepared fresh for each use.

### Product Description

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). 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 at 560 nm (Figure 1).

However, the light production resulting from the reaction leads to formation of suicidal adenylyl-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 adenylyl-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, allowing batch processing in microplates (Figure 2).

Glow-type luciferase assays like Steady-Luc have lower luminescence signal compared to flash-type assays. The sensitivity and limit of detection of the assay will depend on luciferase expression levels in your experimental system as well as luminometer sensitivity.

### References

1. Alam, J. and J.L. Cook. 1990. *Anal. Biochem.* 188:245-254.
2. Bronstein, I., et al. 1994. *Anal. Biochem.* 219:169-181.
3. Gould, S.J. and S. Subramani. 1988. *Anal. Biochem.* 175:5-13.
4. Brasier, A.R., et al. 1989. *BioTechniques.* 7:1116-1122.
5. Wood, K.V. 1990. Bioluminescence and Chemiluminescence current status. Proceedings of the VIth International Symposium on Bioluminescence and Chemiluminescence, Cambridge, Ed. By P. Stanley and L. J. Kricka. p543.
6. Airth, R.L., et al. 1958. *Biochimica et Biophysica Acta* 27:519-532.

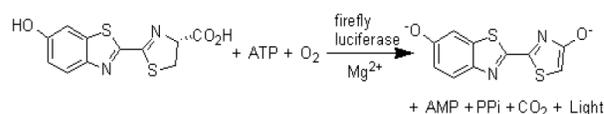


Figure 1. Bioluminescent reaction catalyzed by firefly luciferase.

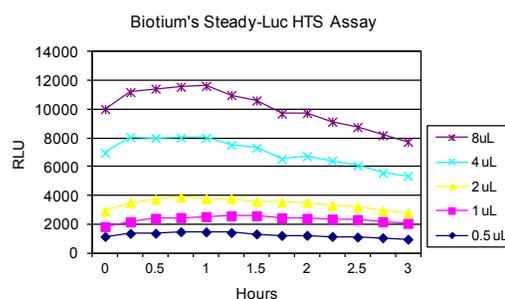


Figure 2: Luminescence kinetics of the Steady-Luc assay. PC3 cells in a 96 well plate were transfected with 0.5, 1, 2, 4 or 8 uL per well of transfection mixture containing firefly luciferase plasmid and Fugene 6 (Roche). 24 hours after transfection, 100 uL of Firefly Steady-Luc Assay Solution was added to each well. After 5 minutes to allow completion of cell lysis, the 96-well plate was then placed in a MicroLumi96 Microplate Luminometer (Harta Instruments) for measurement of relative luminescence units (RLU).

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## Assay Procedure

Note: Luminescence signal has a half-life of 3-5 hours, but may fluctuate over time or with temperature variation, and may vary depending on culture medium used. Therefore, raw luminescence values should be directly compared only for samples in the same medium. For comparison of luminescence signal between plates that are read at different times, each plate should include the same common internal control. The luminescence signals from each plate can be normalized to the internal control from the same plate.

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°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 plates containing mammalian cells from the incubator. If plates will be read in luminescence microplate reader, make sure plates are compatible with the instrument.

4. Add a volume of assay solution equal to that of the culture medium in each well and mix well. For example, for 96-well plates, add 100 uL assay solution to each well containing 100 uL of cells in medium.

5. Wait at least 5 minutes for complete lysis of the cells.

6. Mix samples thoroughly, then measure luminescence with a microplate luminometer. Alternatively lysates can be transferred to tubes for measurement in a single sample luminometer.

## Related Products

Catalog number	Product
30003	Firefly Luciferase Assay Kit
30075	Firefly Luciferase Assay Kit (Lyophilized)
30004	Renilla Luciferase Assay Kit
30005	Firefly & Renilla Dual Luciferase Assay Kit
30020	ATP-Glo Bioluminometric Cell Viability Assay
22003	Mini Cell Scrapers, pack of 200

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