Luciferase Reporter HTS Assay Kit (Firefly)

PromoKine

Instruction Manual

Product Name	Product Description	Size	Catalog Number
Luciferase Reporter HTS Assay Kit (Firefly)	Luciferase Reporter HTS Assay Kit (Firefly)	4 ml 12 ml 100 ml 10 x 100 ml	PK-CA707-30028-0 PK-CA707-30028-1 PK-CA707-30028-2* PK-CA707-30028-3*

*sizes currently not available

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).

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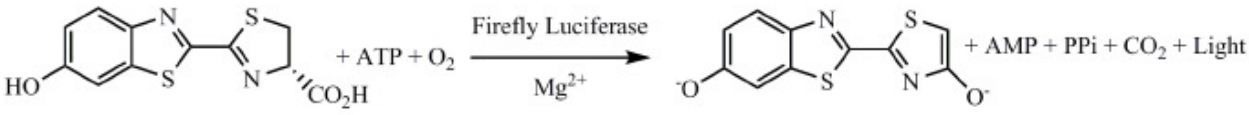
However, the light production resulting from the reaction leads to formation of suicidal adenyl-oxyluciferin at the enzyme surface. It results in very short half-life of the light emission with a flash-type kinetics. Several substances have been described to prolong light production by regenerating enzyme through removing inhibitory oxyluciferin from the enzyme surface (5, 6), but the signal duration (10-15 minutes) is still too short for batch process screening.
PromoKine's Luciferase Reporter HTS sssay 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 for the quantification of firefly luciferase expression in mammalian cells with signal half life of about 3 hours (Figure 2). Glow-type luciferase assays like this one 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.

Figure 1: Bioluminescent reaction catalyzed by Firefly luciferase.



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PromoKine's Luciferase Reporter HTS 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).

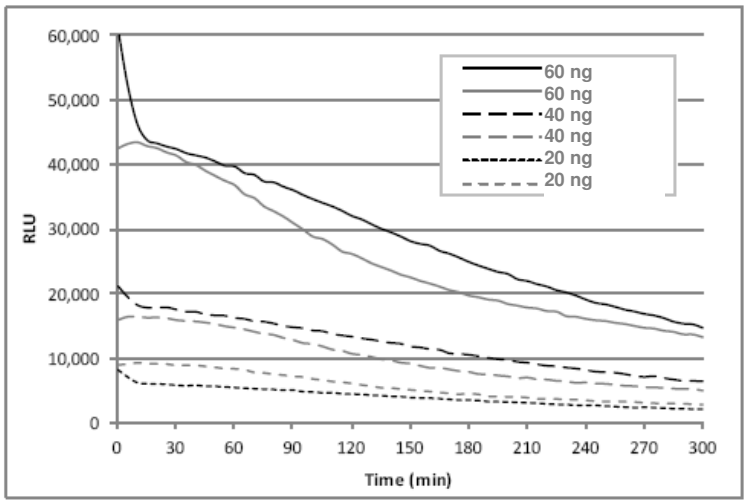

Figure 2: Reporter gene assays with transfected CHO-K1 cells using PromoKine's Luciferase Reporter HTS Assay Kit (Firefly). CHO-K1 cells were grown in F12-K medium containing 10% FBS in a white 96-well plate. On the day after plating, cells were transiently transfected with varying amounts of firefly luciferase expression vector. On the day after transfection, the medium was replaced with fresh growth medium (100 µl per well). The plate was allowed to equilibrate to room temperature, and 100 µl Assay Buffer containing D-Luciferin was added to each well. The plate was placed in a Bio-Tek Synergy H1 microplate reader and mixed with fast orbital shaking for five minutes. Luminescence was read every five minutes for five hours, with three seconds of orbital shaking before each read. Background luminescence values from untransfected cells were subtracted from luminescence values for each time point. Averaged luminescence values for duplicate wells are shown for various amounts of cells transfected with the luciferase reporter vector (ng/per well).

Kit Contents

Components:	PK-CA707-30028-0	PK-CA707-30028-1	PK-CA707-30028-2	PK-CA707-30028-3
	40 assays[0]	120 assays[1]	1,000 assays[2]	10,000 assays[3]
D-Luciferin	1 mg	3 x 1 mg	25 mg	10 x 25 mg
Firefly Assay Buffer	4 ml	12 ml	100 ml	10 x 100 ml

[0]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 40, 160 and 1,320 assays respectively.

[1]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.

[2]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.

[3]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 and Stability

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

Intended Use

For in vitro research use only. Not for diagnostic or therapeutic procedures.

Assay Protocol

Note: The luminescence signal generated using this kit has a half-life of about 3 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.

The assay should be carried out on cells or samples in cell culture medium containing magnesium. Luminescence signal will be low in the absence of magnesium.

Assay Procedure

1. Equilibrate the kit components to room temperature (22°C) before reconstitution.
   To prepare *Firefly Working Solution*, mix lyophilized D-Luciferin substrate and Firefly Assay Buffer in 1 mg to 4 ml ratio. For each 1 mg vial of lyophilized substrate, mix with 4 ml Firefly Assay Buffer. For each 25 mg vial lyophilized substrate, mix with 100 ml Firefly Assay Buffer. Mix well the contents of the vial by inversion until the substrate is completely dissolved. It is recommended to add a small volume of Assay Buffer to the D-luciferin vial and mix by inversion until the substrate is completely dissolved, then transfer the D-luciferin solution to the full volume of Assay Buffer required. Only prepare reagents as needed for one day.
   Note: D-luciferin in Assay Buffer has limited stability. Instead of dissolving the entire contents of the D-luciferin vial in Assay Buffer, you may prepare a D-luciferin stock solution at 10 mg/ml in dH_2O, 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 D-luciferin in Assay Buffer to a final concentration of 0.25 mg/ml (2.5 µl of 10 mg/ml D-luciferin stock solution per 100 µl assay buffer).
2. Remove plates containing luciferase-expressing cells from the incubator. If plates will be read in luminescence microplate reader, make sure plates are compatible with the instrument.
3. Add a volume of *Firefly Working Solution* equal to that of the culture medium in each well and mix well. For example for 96-well plates: add 100 µl *Firefly Working Solution* to each well containing 100 µl of cells in medium for a final volume of 200 µl per well. 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.
4. Wait at least 5 minutes for complete lysis of the cells, then measure luminescence with a microplate luminometer. Mixing on an orbital shaker to enhance cell lysis is recommended.
5. Immediately before reading luminescence, mix samples thoroughly. Measure luminescence with a microplate luminometer. Alternatively, cell lysates can be transferred to tubes to be measured in a single sample luminometer.

References

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11/2015