Luciferase Reporter Assay Kit I (Firefly)

PromoKine

Instruction Manual

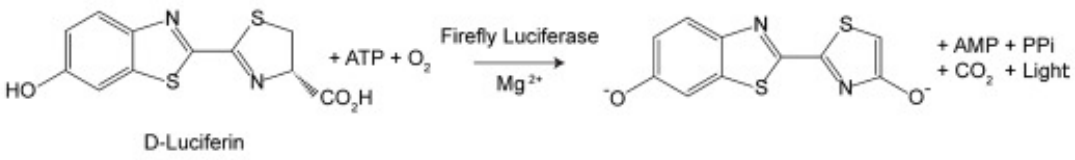
Product Name	Product Description	Size	Catalog Number
Luciferase Reporter Assay Kit I (Firefly)	Luciferase Reporter Assay Kit I (Firefly)	150 assays 1000 assays	PK-CA707-30003-1 PK-CA707-30003-2

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). As with many enzymes, *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 Luciferase Reporter Assay kit is designed for detection and quantification of the *Firefly* luciferase reporter enzyme from cultured cells in a simple, efficient, and linear fashion (Figure 2).

This is a flash-type luminescence assay that requires signal to be measured immediately after adding working solution to samples. The luminescence signal decays over the course of about 10 minutes of reaction time (Figure 3), although signal half-life may vary depending on luciferase expression levels. PromoKine also offers the Firefly Luciferase Reporter HTS Kit (cat. no. PK-CA707-30028), which is a homogenous glow-type assay with signal half-life of 3-5 hours (see website).

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



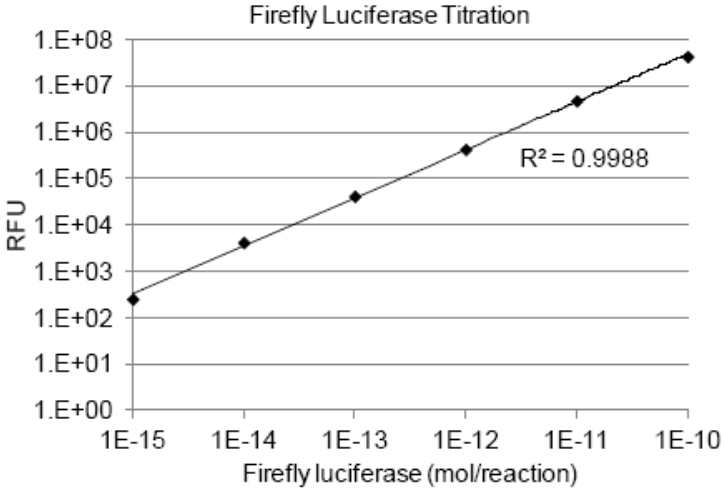


Figure 2. Titration of recombinant firefly luciferase in the Firefly Luciferase Assay.
Quantilum® Luciferase (Promega) was serially diluted in 1X Firefly Lysis Buffer with 1 mg/mL BSA and measured in the assay. Luminescence was measured on a Promega Glomax® 20/20 single tube luminometer with integration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

Kit Contents

Components:	PK-CA707-30003-1	PK-CA707-30003-2
	150 assays	1,000 assays
D-Luciferin	3 vials (1 mg each)	2 vials (10 mg each)
Firefly Luciferase Assay Lysis Buffer (5X)	15 ml	2 x 15 ml
Firefly Luciferase Assay Buffer	15 ml	100 ml

Note: Enough lysis buffer is provided to perform the stated number of assays with cells grown in culture plate sizes ranging from 96-well to 24-well. For applications requiring more lysis buffer (see Assay Protocol), additional 5X passive lysis buffer (cat. no. PK-CA707-99912) may be purchased separately.

Storage and Stability

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.

Assay 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 de-ionized water and mixing well. The 1X Lysis Buffer may be stored at 4°C for up to one month. Store the 5X *Firefly* Luciferase Lysis Buffer at –20°C.

B. Lysis of Cells Cultured in Multiwell Plates

1. Remove growth medium from 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 *Firefly* Luciferase Lysis Buffer to each well using the volume recommended below for each type of culture plate:
    - 6 well culture plate: 500 µl per well
    - 12 well culture plate: 250 µl per well
    - 24 well culture plate: 100 µl per well
    - 48 well culture plate: 65 µl per well
    - 96 well culture plate: 20 µl per well
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. PromoKine offers mini cell scrapers (Cat.No. PK-CA707-22003) for harvesting lysates from 96-, 48-, and 24-well plates.

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

Firefly Luciferase Assay

A. Preparation of *Firefly* Luciferase Working Solution

1. Thaw a bottle of *Firefly* Luciferase Assay Buffer at room temperature.
2. Prepare 10 mg/mL D-luciferin stock solution. For 1 mg D-luciferin, add 100 µL water to the vial and mix. For 10 mg D-luciferin, 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 µL working solution per assay). Add D-luciferin (10 mg/mL) to assay buffer at a ratio of 1:50. For example, add 20 µL 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

The protocol below is for manual assay using a single-tube luminometer. If your luminometer is equipped with automatic injectors, they may be used to dispense working solution into each luminometer tube or well of a multiwell plate according to the instructions for your instrument.

For manual luminometer:

1. Set up luminometer with parameters recommended for your instrument. 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 µl of *Firefly* Luciferase 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 (protocol example; please adapt protocol to instructions of your instrument):

1. Format the luminometer so that the injector dispenses 100 µl. Prime the injector with *Firefly* Luciferase Assay Buffer.
2. For each reaction, carefully add 20 µl 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 Working 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.

Intended Use

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

References

1. Alam, J. and J.L. Cook. 1990. Reporter genes: Application to the study of mammalian gene transcription. Anal. Biochem. 188:245-254.
2. Bronstein, I., et al. 1994. Chemiluminescent and bioluminescent reporter gene assays. Anal. Biochem. 219:169-181.
3. Gould, S.J. and S. Subramani. 1988. *Firefly* luciferase as a tool in molecular and cell biology. Anal. Biochem. 175:5-13.
4. Brasier, A.R., et al. 1989. Optimized use of the *Firefly* luciferase assay as a reporter gene in mammalian cell lines. BioTechniques. 7:1116-1122.

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03/2017