



Firefly Luciferase Activity Assay Kit

Item No. 702710

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage Temperature
401069	Firefly Luciferase Assay Buffer	5 vials/15 ml	-20°C
401066	Firefly Luciferase Substrate Mix	5 vials	-20°C

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.



WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's Firefly Luciferase Activity Assay Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. Mammalian cells expressing firefly luciferase
2. An orbital microplate shaker
3. A plate reader capable of measuring luminescence
4. Adjustable pipettes; multichannel or repeating pipettor recommended
5. Materials needed for cell culture
6. White 96- or 384-well plates

INTRODUCTION

Background

Firefly luciferase (Fluc) is a commonly used enzymatic reporter that catalyzes the oxidation of D-luciferin in the presence of ATP, generating a luminescent signal that can be detected with a luminometer.^{1,2} Fluc has high specificity, no endogenous activity, and a wide dynamic range, making it an ideal reporter. Luciferase reporter assays have been widely used to determine transcriptional promoter activity in response to cell signaling. Luciferase mRNA is also commonly used as a reporter to assess the delivery efficiency and tissue distribution of lipid nanoparticle (LNP) formulations.³

About This Assay

Cayman's Firefly Luciferase Activity Assay Kit provides a convenient and sensitive method for detecting firefly luciferase activity in user-supplied cultured mammalian cells, such as those used in luciferase reporter assays. The included assay buffer efficiently lyses cells, enabling the luciferase enzyme to access D-luciferin and ATP present in the Firefly Luciferase Substrate Mix to produce a luminescent signal (Figure 1, on page 7). The signal intensity is directly proportional to the level of active firefly luciferase present in the sample.

This kit contains a sufficient volume of reagents to assay 160 samples in triplicate using a 96-well plate format or 640 samples in triplicate using a 384-well plate format.

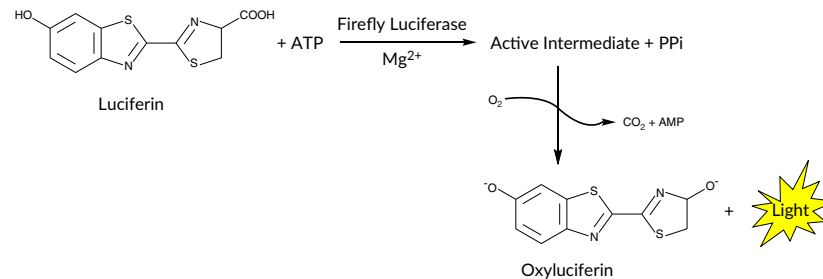


Figure 1. Assay scheme

PRE-ASSAY PREPARATION

Reagent Preparation

1. Firefly Luciferase Assay Buffer – (Item No. 401069)

Each vial contains 15 ml of Firefly Luciferase Assay Buffer. Once thawed, mix by gently inverting the vial prior to use. Let the buffer equilibrate to room temperature before reconstituting the Firefly Luciferase Substrate Mix (see below).

2. Firefly Luciferase Substrate Mix – (Item No. 401066)

Each vial contains lyophilized ATP and D-luciferin. Immediately prior to use, reconstitute the contents of each vial with 4 ml of assay buffer before transferring to a larger container and diluting with an additional 8 ml of assay buffer. Mix thoroughly. *Do not vortex*. One vial provides a sufficient volume to assay a full plate. If additional wells will be utilized, reconstitute additional vials. The reconstituted substrate mix will be stable for one day when stored at 4°C protected from light.

ASSAY PROTOCOL

Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (i.e., slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The Firefly Luciferase Substrate Mix should be prepared as described in the **Reagent Preparation** section (see page 8) and protected from light.
- The assay is performed at room temperature (22-25°C).
- When assaying in triplicate, a total of 160 samples or 640 samples can be measured on 96-well plates or 384-well plates, respectively.

Performing the Assay

NOTE: Cells expressing firefly luciferase should be cultured and prepared according to the experimental protocol being evaluated. Cell seeding density, transfection conditions, treatment conditions, and incubation times should be optimized for the cell type and experimental application.

1. Culture cells expressing firefly luciferase in white opaque 96- or 384-well plates and treat according to the reporter assay, transfection, or other experimental protocol being evaluated. The sample volume should not exceed 100 μ l in a 96-well plate or 25 μ l in a 384-well plate.
2. After treatment, do not aspirate the cell culture supernatant. Instead, add an equal volume of reconstituted Firefly Luciferase Substrate Mix directly to the wells. The substrate mix lyses the cells and initiates the reaction.
3. Mix gently on an orbital plate shaker, taking care to avoid the formation of bubbles. Incubate for 20 minutes at room temperature protected from light.
4. Measure luminescence.

ANALYSIS

Calculations

Luciferase activity can be normalized to an internal reference, such as cell number or a co-transfected control reporter. Once normalized, the activity of each sample can be expressed relative to a control by dividing the luminescence of the sample by the luminescence of the control.

Example Data

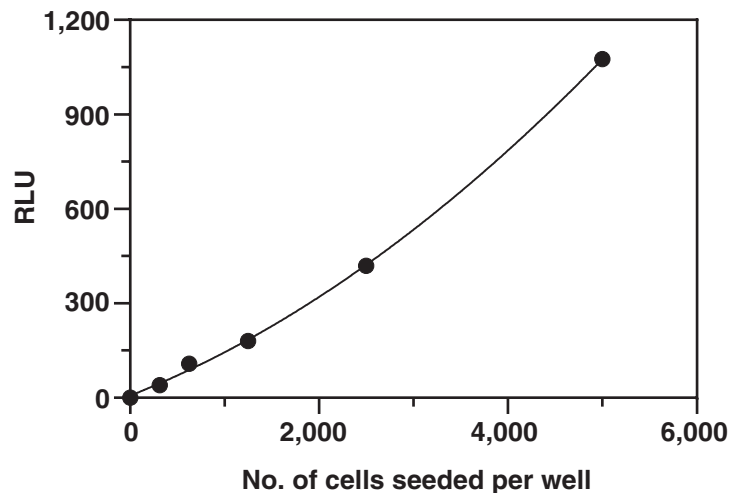


Figure 2. Detection of bioluminescence following transfection with firefly luciferase mRNA via LNPs. Varying numbers of A549 cells in DMEM were seeded in the wells of a 96-well plate and transfected using SM-102 LNPs containing 30 ng of Firefly Luciferase mRNA (Item No. 39801) per well. After 48 hours of incubation, the expression of luciferase was detected using Cayman's Firefly Luciferase Activity Assay Kit.

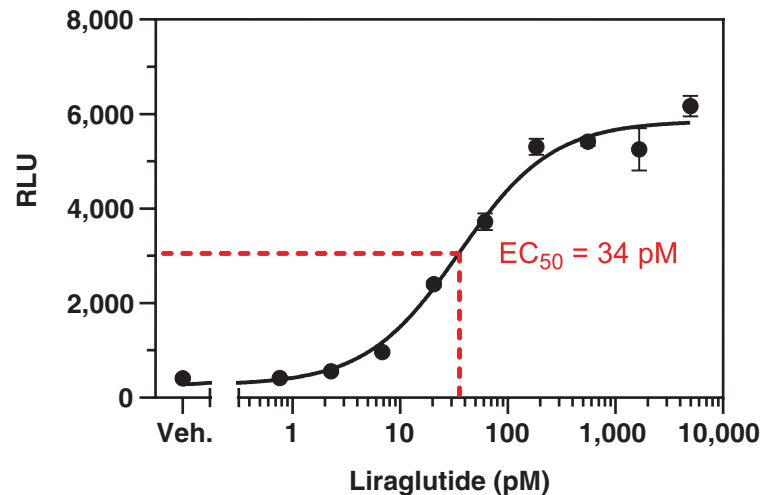


Figure 3. Detection of firefly luciferase activity in a GLP-1 receptor reporter assay. The luciferase reporter gene, controlled by a CREB-responsive promoter, was used to monitor glucagon-like peptide 1 receptor (GLP-1R) activation in HEK293T/17 cells. Cells were seeded at 40,000 cells/well and incubated overnight. The next day, the cells were treated with different doses of liraglutide (Item No. 24727) in serum-free medium or with serum-free medium as a vehicle control (Veh). After six hours of stimulation, luciferase activity was detected using Cayman's Firefly Luciferase Activity Assay Kit.

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of replicates	A. Poor pipetting/technique B. Bubble in the well	A. Use a multi-channel pipette B. Carefully tap the side of the plate to remove bubbles
No activity detected in sample wells	Firefly luciferase expression level is too low	A. Increase the <i>gain</i> or integration time and re-read B. Repeat the experiment with a higher number of cells per well

References

1. Zhang, R. and Zheng, H. Luciferase reporter assay for determining the signaling activity of interferons. *Methods Mol. Biol.* **2854**, 19-28 (2025).
2. Müller, K., Ogris, M., Sami, H. Firefly luciferase-based reporter gene assay for investigating nanoparticle-mediated nucleic acid delivery. *Methods Mol. Biol.* **1943**, 227-239 (2019).
3. Kang, D.D., Marks, A., Morla-Folch, J., *et al.* Targeting and tracking mRNA lipid nanoparticles at the particle, transcript and protein level. *Nat. Biomed. Eng.* **9**, 1591-1609 (2025)

Assay Summary

Item No.	Reagent	Procedure
401069	Firefly Luciferase Assay Buffer	Thaw before using, equilibrate to room temperature
401066	Firefly Luciferase Substance Mix	Reconstitute with 12 ml of Firefly Luciferase Assay Buffer

Table 1. Reagent preparation summary

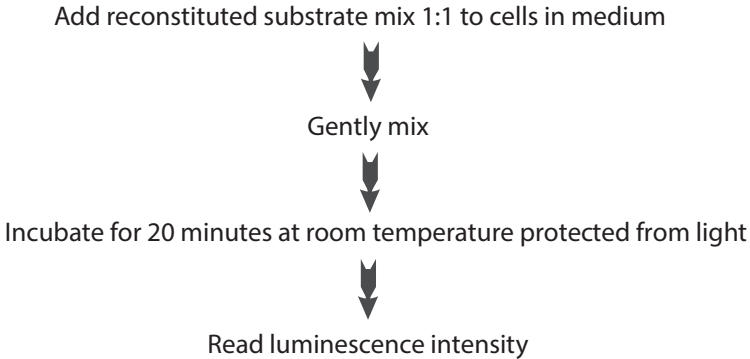
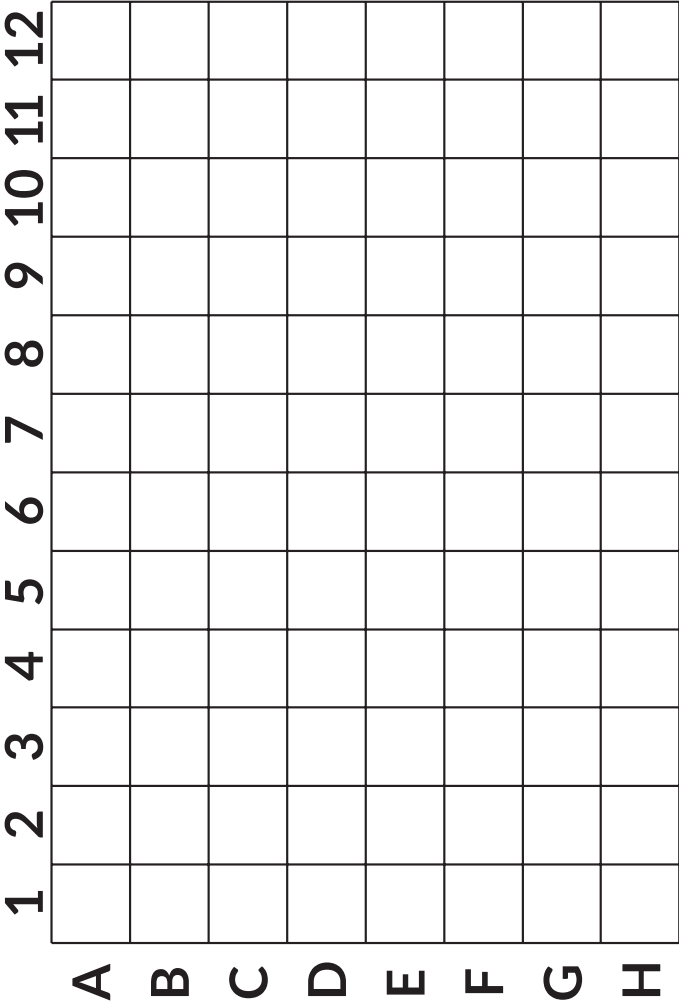


Figure 4. Assay summary



Warranty and Limitation of Remedy

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