Firefly Luciferase Assay Kit

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1. Introduction

Firefly luciferase is widely used as a reporter for studying gene regulation and function, and for pharmaceutical screening. It is a very sensitive genetic reporter due to the absence of endogenous luciferase activity in mammalian cells or tissues. Firefly luciferase is a 62KDa protein, which is active as a monomer and does not require subsequent processing for its activity. The enzyme catalyzes ATP-dependent D-luciferin oxidation to oxyluciferin, producing light emission centered at 560 nm. 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 firefly luciferase assay kit is designed for simple and efficient quantitation of firefly luciferase reporter enzyme activity from cultured cells with high sensitivity and linearity. 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, although signal half-life may vary depending on luciferase expression levels.

2. Products

Kit Components

Components	PR300001 (150 assays)	PR300006 (1000 assays)
5X Firefly Luciferase Lysis Buffer	15 mL	2 X 15 mL
Firefly Luciferase Assay Buffer 2.0	15 mL	100 mL
D-Luciferin	3 x 1 mg	2 x 10 mg

Note: Sufficient firefly lysis buffer is provided to perform the stated number of assays with cells grown in 96 well plates. For applications requiring more lysis buffer (e.g. >100 uL/well), additional 5X lysis buffer (Cat. # PR300005) may be purchased separately

Storage and Handling

Store the kit at –20°C or below. Firefly Luciferase Assay Buffer 2.0 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.

Notice to purchaser

This product is for research use only. Use in and/or for diagnostics and therapeutics is strictly prohibited. By opening and using the product, the purchaser agrees to the following: The product may not be distributed, resold, modified for resale or used to manufacture commercial products without prior written approval from OriGene Technologies, Inc. If you do not agree to the above conditions, please return the

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UNOPENED product to OriGene Technologies, Inc. within ten (10) days of receipt for a full refund by contacting customer service at custsupport@origene.com.

3. Experimental protocol

Preparation of Cell Lysates

1. Prepare 1X Firefly Luciferase lysis buffer by adding 1 volume of 5X Firefly Luciferase Lysis Buffer to 4 volumes of dH2O and mixing well. 1X lysis buffer may be stored at 4°C for up to one month. Store 5X firefly luciferase lysis buffer at –20°C.

2. Remove the growth medium from the 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 passive lysis buffer using the volume recommended below for each type of well:

Wells/plate	Lysis buffer/well
6 well	500 uL
12 well	250 uL
24 well	100 uL
48 well	65 uL
96 well	20 uL

3. 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 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.

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

Preparation of Firefly Working Solution

1. Thaw Firefly Luciferase Assay Buffer 2.0 at room temperature.

2. Prepare 10 mg/mL D-luciferin stock solution. For 1 mg D-luciferin (from Cat# PR300001), add 100 uL water to the vial and mix. For 10 mg D-luciferin (from Cat# PR300006), add 1mL 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.

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3. Prepare enough firefly working solution to perform the desired number of assays (100 uL working solution per assay). Add D-luciferin (10 mg/mL) to assay buffer at a ratio of 1:50. For example, add 20 uL 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.

Firefly Luciferase Assay

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 multi-well plate according to the instructions for your instrument.

1. Set up luminometer with parameters recommended for your instrument. We routinely use integration time of 1 second.

2. Add 20 uL of cell lysate into a reaction tube that is compatible with your luminometer.

3. Add 100 uL of firefly working solution to the reaction tube and mix by pipetting or vortexing.

4. Immediately place tube in luminometer and record the firefly luminescence measurement.

Determination of Assay Background

The expression of a luciferase reporter is quantified by the luminescence produced above background levels. In most cases, background created by the reagent in the absence of luciferase is very low compared to signal with luciferase. However, when measuring low levels of luciferase activity, it is important to subtract the background signal from untransfected cells or cells transfected with a negative control vector from measurements of luciferase activity.

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