

Illumination™ Firefly Luciferase Enhanced Assay Procedure for Luciferase Reporter Assay

Introduction

Firefly Luciferase Assays are one of the leading reporter assays in the world in the measurement of gene function and gene regulation as well as being widely used in pharmaceutical screening. The luciferase assays are sensitive and convenient due to the absence of endogenous luciferase activity in most cell types and tissue. Firefly luciferase is a monomeric 62 kDa protein typically isolated from the firefly, *Photinus pyralis*, which catalyzes the ATP-dependent D-luciferin in the presence of oxygen and Mg^{2+} to oxyluciferin to produce a yellow to greenish light (~560 nm).

GoldBio's Illumination™ Firefly Luciferase Enhanced Assay Kit is specifically designed for the simple and efficient quantification of firefly luciferase reporter enzyme activity from cultured cells with the highest degree of sensitivity, reliability and linearity. This kit is a flash-type luminescent assay, and requires measurement immediately after adding the substrate to the sample. Our Enhanced Assay Kit includes our improved 5X Luciferase Lysis Buffer which provides a better working solution stability compared to other firefly assay kits. The luminescence signal decreases about 50% after about 10 minutes of reaction time (Figure 2), although signal half-life may vary depending on luciferase expression levels.

Materials

Table 1. Kit Components

Component	I-930-50 (50 assays)	I-930-150 (150 assays)	I-930-1000 (1000 assays)
5X Luciferase Lysis Buffer	5 ml (Catalog # L-740)	15 ml (Catalog # L-740)	2 x 15 ml (Catalog # L-740)
Firefly Luciferase Assay Buffer	5 ml 50 assays	15 ml 150 assays	100 ml 1000 assays
GoldBio D-Luciferin	1 x 1 mg (Catalog # LUCK/LUCNA)	3 x 1 mg (Catalog # LUCK/LUCNA)	2 x 10 mg (Catalog # LUCK/LUCNA)

Note: Sufficient firefly lysis buffer is provided to perform the stated number of assays with cells grown in 96–24 well plates. For applications requiring more lysis buffer (e.g. >100 μ l/well), additional 5X Luciferase Lysis Buffer ([Catalog # L-740](#)) may be purchased separately.

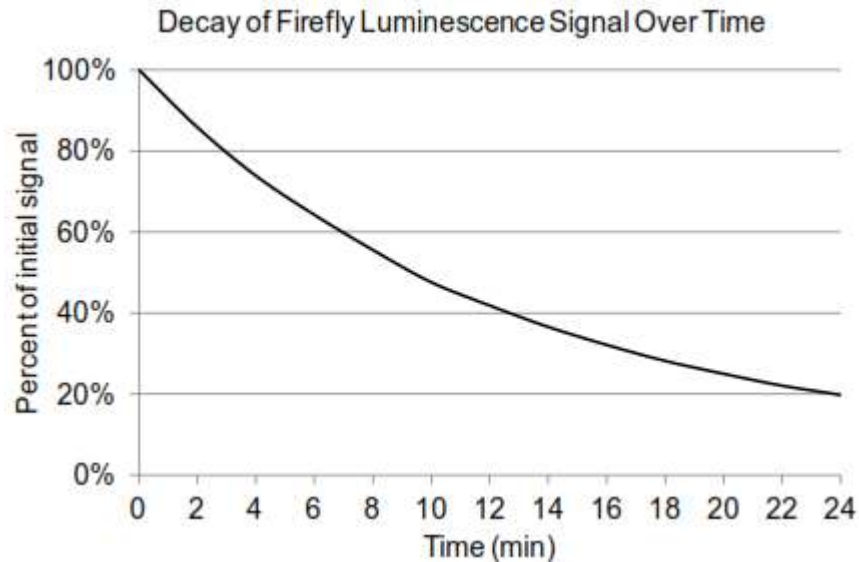
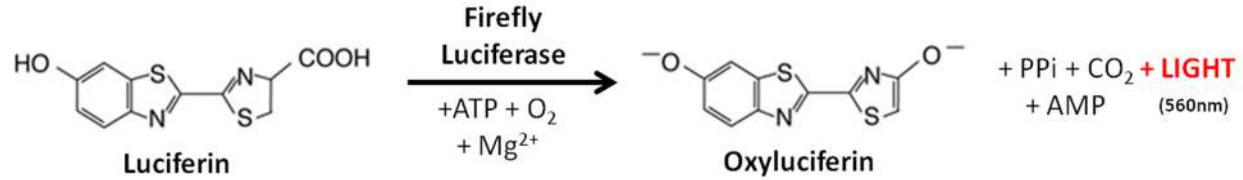


Figure 2. Stability of luminescence signals in the Illumination™ firefly luciferase enhanced assay. Luminescence measurements were carried out in a white 96-well plate on cells transfected with firefly luciferase. Luminescence was measured using a Bio-Tek H1m microplate reader every 2 minutes for 24 minutes, and RLU values were normalized to the first measurement for each reaction.

Storage/Handling

Store the kit at -20°C. Firefly Luciferase Assay Buffer is stable at -20°C for three months and is stable 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.

Method

Preparation of Cell Lysates

1. Preparation of Firefly Luciferase Lysis Buffer
 - a. 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 luciferase lysis buffer at -20°C.
2. Lysis of Cells Cultured in Multiwell Plates
 - a. Remove growth medium from cultured cells and gently add a sufficient volume of phosphate buffered saline (PBS) ([GoldBio Catalog # P-271](#)) to wash the surface of the culture vessel. Remove the PBS and add 1X firefly lysis buffer to each well using the volume recommended below for each type of culture plate:

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

- b. 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 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. See GoldBio's [Luciferin In Vitro Handbook](#) for more tips and suggestions.

- c. Transfer the lysate to a tube or vial. Place at 4°C until ready to assay. Store lysates at -20°C or -80°C if assay will not be performed on the same day.

Note: (Optional) The lysate can be cleared by centrifugation for 30 seconds at top speed in a refrigerated microcentrifuge and transferred into a new tube.

Preparation of Firefly Luciferase Working Solution

1. Thaw Firefly Luciferase Assay Buffer at room temperature.
2. Prepare 10 mg/ml D-luciferin stock solution. For **I-930-50** or **I-930-150**, add 100 µl dH₂O to the vial (1 mg) and mix. For **I-930-1000**, add 1 ml dH₂O to the vial (10 mg) 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 luciferase 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 (luciferase 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

1. For manual luminometer:
 - a. Set up luminometer with appropriate parameters (delay time, integration time, sensitivity, etc.).
 - b. Add 100 µl of firefly luciferase working solution to the luminometer tube.
 - c. Add 20 µl of cell lysate and mix quickly by vortexing or flicking the tube with a finger.
 - d. Place tube in luminometer and initiate measurement. Luminescence is normally integrated over 10 seconds without delay. Other integration times may also be used.
 - e. If the luminometer is not connected to a printer or computer, record the firefly luciferase activity measurement.
 - f. Discard the reaction tube, and proceed to the next firefly luciferase reaction.
2. For luminometer with injector:
 - a. Format the luminometer so that the injector dispenses 100 µl. Prime the injector with firefly luciferase working solution.
 - b. For each reaction, carefully add 20 µl of cell lysate to an individual luminometer tube or to the wells of a multiwell plate.
 - c. Place the samples in a luminometer.

- d. Initiate measurement. This 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 also may be used.
- e. Record the firefly luciferase activity measurement.
- f. If using a single tube luminometer, discard the reaction tube, and proceed to the next firefly luciferase reaction. If using a plate luminometer, the luminometer will automatically begin injecting firefly luciferase working solution into the next well indicated on the luminometer plate.

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.

Associated Products

GoldBio Catalog #	Product Name
I-935	Illumination™ Lyophilized Firefly Luciferase Enhanced Assay Kit
I-940	Illumination™ Firefly Luciferase Stabilizer
I-945	Illumination™ Dura-Luc Firefly HTS Assay Kit
I-946	Illumination™ Dura-Luc Lyophilized Firefly HTS Assay Kit
LUCK	D-Luciferin, Potassium Salt (Proven and Published™)
LUCNA	D-Luciferin, Sodium Salt (Proven and Published™)
L-740	5X Luciferase Lysis Buffer
I-920	Illumination™ Firefly & Renilla Luciferase Enhanced Assay Kit

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