Protocol

TD-P Revision 2.0

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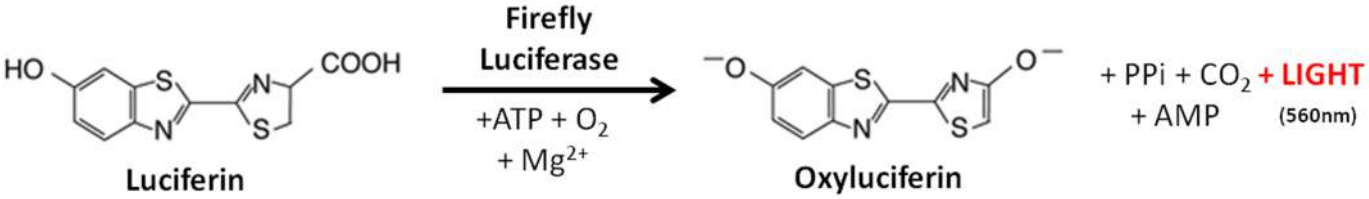
Illumination™ Lyophilized Firefly Luciferase Enhanced Assay

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 producing a yellow to greenish light (~560 nm).

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. The Firefly Assay buffer is packaged as a lyophilized powder, which can be shipped at ambient temperature and stored at -20°C instead of -80°C. This is a flash-type luminescence assay with signal half-life of about 12 minutes.



Materials

Table1. Kit Components

Component	I-935-150 (150 assays)	I-935-1000 (1000 assays)
5X Luciferase Lysis Buffer	15 ml (Catalog # L-740)	2 x 15 ml (Catalog # L-740)
Firefly Luciferase Assay Buffer (lyophilized)	150 assays*	1000 assays*
GoldBio D-Luciferin	3 x 1 mg (Catalog # LUCK/LUCNA)	2 x 10 mg (Catalog # LUCK/LUCNA)

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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 µl/well), additional 5X Luciferase Lysis Buffer (Catalog # L-740) may be purchased separately.
***See protocol for instructions for dissolving lyophilized buffer.**

Storage/Handling

Store the kit at -20°C. The kit is stable at -20°C for at least six months from date of receipt. After reconstitution, aliquot Firefly Luciferase Assay Buffer, if necessary, to avoid repeated freeze-thaw cycles; reconstituted Luciferase Assay Buffer is stable at -80°C for at least 6 months. Firefly luciferase working solution (assay buffer + D-luciferin) should be prepared fresh on the day of assay.

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 dH_2O 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. 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. Reconstitute the firefly luciferase assay buffer by adding the appropriate volume of dH_2O to the bottle. For **I-935-150**, add 15 ml dH_2O. For **I-935-1000**, add 100 ml dH_2O. Mix gently by rocking or inverting until the lyophilized buffer has completely dissolved into a homogenous solution.

   Note: See Storage/Handling for storage of unused firefly luciferase assay buffer after reconstitution.

2. Prepare an adequate volume of working solution to perform the desired number of firefly luciferase assays (100 µl working solution per assay). Thaw a bottle of firefly luciferase assay buffer and pipette a desired volume (5 ml or 50 ml) from the bottle into a clean container.

3. Dissolve the supplied D-luciferin in the firefly luciferase assay buffer from step 1 at a final concentration of 0.2 mg/ml. For kit **I-935-150**, dissolve one vial of D-luciferin (1 mg/vial) in 5 ml assay buffer. For kit **I-935-1000**, dissolve one vial of D-luciferin (10 mg/vial) in 50 ml assay buffer. Firefly luciferase working solution (D-luciferin + firefly luciferase assay buffer) should be prepared fresh and used within a day.

   Note: D-Luciferin in assay buffer has limited stability. If you need less than 5 mL or 50 mL luciferase working solution as described in step 2, you may dissolve D-luciferin in dH_2O as 10X or 50X stock solution and store it in aliquots 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 the stock solution in firefly luciferase assay buffer to a final concentration of 0.2 mg/ml D-luciferin.

Standard Reporter Protocol

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.

Associated Products

GoldBio Catalog #	Product Name
I-930	Illumination™ 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