Firefly Luciferase Assay

Cell Based Assay

Cat. # SCT154

pack size: 1 Kit

Store at -80ºC

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Data Sheet

Background

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 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 to oxyluciferin, producing light emission centered at 560 nm. Light emitted from the reaction is directly proportional to the number of luciferase enzyme molecules.

The Firefly Luciferase Assay 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.

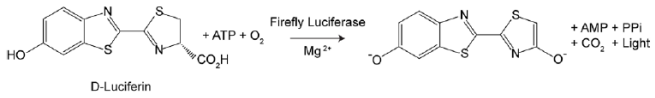

Figure 1. Assay principle. Bioluminescent reaction catalyzed by firefly luciferase.

Storage

Store Firefly Luciferase Assay at -80°C. 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.

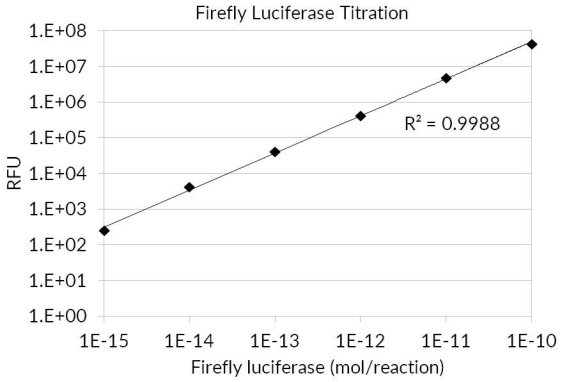

Figure 2. Titration of recombinant firefly luciferase in the Firefly Luciferase Assay. Recombinant luciferase was serially diluted in 1X Firefly Lysis Buffer with 1 mg/mL BSA and measured in the assay. Luminescence was measured on a Promega Glomax® 20/20 single tube luminometer with integration time of 1 second.

Kit Components

1) 5X Firefly Luciferase Lysis Buffer (CS224525): 2 X 15 mL

2) Firefly Luciferase Assay Buffer 2.0 (CS224585): 100 mL

3) D-Luciferin (CS224519): 2 X 10 mg

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Assay Protocol

Preparation of Cell Lysates

Preparation of Firefly Luciferase Lysis Buffer

1. Prepare 1X 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.

Lysis of Cells Cultured in Multiwell Plates

1. 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 lysis buffer using the volume recommended below for each type of well:

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

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 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.

3. 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. Add 1 mL water to the 10 mg 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.

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 multiwell 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 µL of cell lysate into a reaction tube that is compatible with your luminometer.
3. Add 100 µL 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.