Firefly Luciferase Assay (Lyophilized)

Cell Based Assay

Cat. # SCT151

pack size: 1 Kit

Store at -20ºC

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 (Lyophilized) 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 convenient 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.

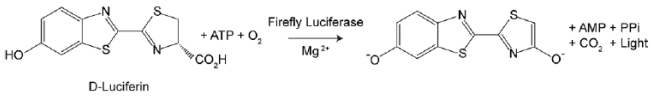

Figure 1. Assay principle. Bioluminescent reaction catalyzed by firefly luciferase.

Storage

Store Firefly Luciferase Assay (Lyophilized) at -20°C. The kit is stable at -20°C for at least six months from date of receipt. After reconstitution, aliquot assay buffer if necessary to avoid repeated freeze-thaw cycles; reconstituted assay buffer is stable at -20°C for at least 3 months or -80°C for at least 6 months. Firefly luciferase working solution (assay buffer + D-luciferin) should be prepared fresh on the day of assay.

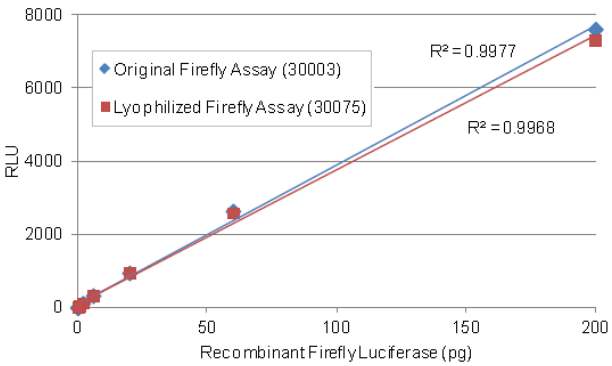

Figure 2. Comparison of dose response curves of 0.02-200 pg Recombinant Firefly Luciferase assayed using Firefly Luciferase Assay (SCT154) or Lyophilized Firefly Luciferase Assay (SCT151). Luminescence was measured on a TD-20/20 luminometer (Turner Designs). Light emission was measured after 30 seconds without initial pre-read delay.

Kit Components

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

2) Firefly Luciferase Assay Buffer (Lyophilized) (CS224526): 1000 assays

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

Rev.1.0/2018-01-29/SCT151/NJA

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. Reconstitute the Firefly Assay Buffer by adding 100 mL dH2O to the bottle. Mix gently by rocking or inverting until the lyophilized buffer has completely dissolved into a homogenous solution.

   Note: see storage and handling (page 1) for storage of unused Firefly Assay Buffer after reconstitution.

2. Prepare an adequate volume of working solution to perform the desired number of firefly luciferase assays (100 uL 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 (dissolve one vial of D-luciferin (CS224519) 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 dH2O 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.

Firefly Luciferase Assay

For manual luminometer:

1. Set up luminometer with appropriate parameters (delay time, integration time, sensitivity, etc.).
2. Add 100 uL of firefly luciferase working solution to the luminometer tube.
3. Add 20 uL of cell lysate and mix quickly by vortexing or flicking the tube with a finger.
4. Place tube in luminometer and initiate measurement. Luminescence is normally integrated over 10 seconds without delay. Other integration times may also be used.
5. If the luminometer is not connected to a printer or computer, record the firefly luciferase activity measurement.
6. Discard the reaction tube, and proceed to the next firefly luciferase reaction.