Firefly Luciferase HTS Assay

Cell Based Assay

Cat. # SCT150

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.

However, the light production resulting from the reaction leads to formation of suicidal adenyl-oxyluciferin at the enzyme surface. It results in very short half-life of the light emission with a flash-type kinetics. The Firefly Luciferase HTS Assay is designed for is a proprietary mixture of substances that modify the enzymatic reaction to produce a long-lasting signal (steady-glow) by preventing the formation of adenyl-oxyluciferin at the enzyme surface. It is a homogeneous high sensitivity firefly luciferase reporter gene assay kit for the quantification of firefly luciferase expression in mammalian cells with signal half-life of about 3 hours (Figure 2). Glow-type luciferase assays have lower luminescence signal compared to flash-type assays. The sensitivity and limit of detection of the assay will depend on luciferase expression levels in your experimental system as well as luminometer sensitivity

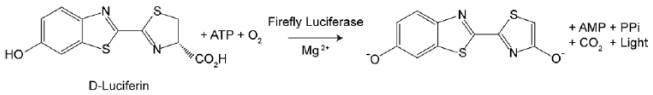

Figure 1. Assay principle. Bioluminescent reaction catalyzed by firefly luciferase.

Storage

Store Firefly Luciferase HTS Assay at -80°C. Kit components are stable for at least six months from date of receipt when stored as recommended. Avoid repeated freeze-thaw cycles.

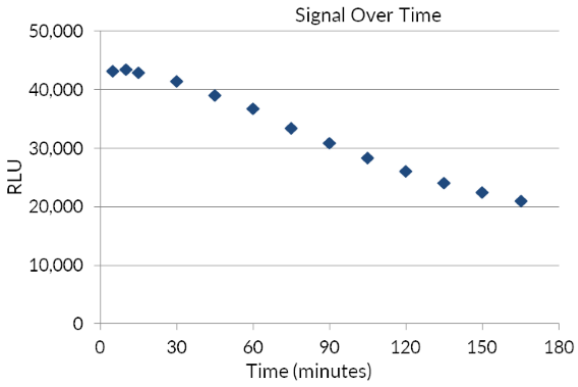

Figure 2. The Firefly Luciferase HTS Assay is a steady-glow high sensitivity firefly luciferase reporter gene assay kit for the quantification of firefly luciferase expression in mammalian cells with signal half life of about 3 hours.

Kit Components

1) Assay Buffer (CS224524): 1X100 ml

2) D-Luciferin (CS224523): 1 X 25 mg

Rev.1.0/2018-01-29/SCT150/NJA

Assay Protocol

Note: The Firefly Luciferase HTS Assay luminescence signal has a half-life of about 3 hours, but may fluctuate over time or with temperature variation, and may vary depending on culture medium used. Therefore, raw luminescence values should be directly compared only for samples in the same medium. For comparison of luminescence signal between plates that are read at different times, each plate should include the same common internal control. The luminescence signals from each plate can be normalized to the internal control from the same plate.

Note: The Firefly Luciferase HTS Assay should be carried out on cells or samples in cell culture medium containing magnesium. Luminescence signal will be low in the absence of magnesium.

1. Equilibrate the kit components to room temperature.

2. To prepare Luciferase working solution, mix D-luciferin substrate and Assay Buffer in 1 mg to 4 mL ratio. For each 1 mg vial of D-luciferin, mix with 4 mL Assay Buffer. For each 25 mg vial of D-luciferin, mix with 100 mL Assay Buffer. Add a small volume of Assay Buffer to the D-luciferin vial and mix by inversion until the substrate is completely dissolved, then transfer the D-luciferin solution to the full volume of Assay Buffer required. Only prepare working solution as needed for one day.

Note: D-luciferin in Assay Buffer has limited stability. Instead of dissolving the entire contents of the D-luciferin vial in Assay Buffer, you may prepare a D-luciferin stock solution at 10 mg/mL in dH2O, and store it at -20oC 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 D-luciferin in Assay Buffer to a final concentration of 0.25 mg/mL (2.5 uL of 10 mg/mL D-luciferin stock solution per 100 uL assay buffer).

3. Remove plates containing luciferase-expressing cells from the incubator. If plates will be read in luminescence microplate reader, make sure plates are compatible with the instrument.

4. Add a volume of assay solution equal to that of the culture medium in each well and mix well. For example, for 96-well plates, add 100 uL assay solution to each well containing 100 uL of cells in medium, for a final volume of 200 uL per well.

5. Wait at least 5 minutes for complete lysis of the cells. Mixing on an orbital shaker during cell lysis is recommended.

6. Immediately before reading luminescence, mix samples thoroughly. Measure luminescence with a microplate luminometer. Alternatively, cell lysates can be transferred to tubes to be measured in a single sample luminometer.