Protocol
Cat. No. K1136

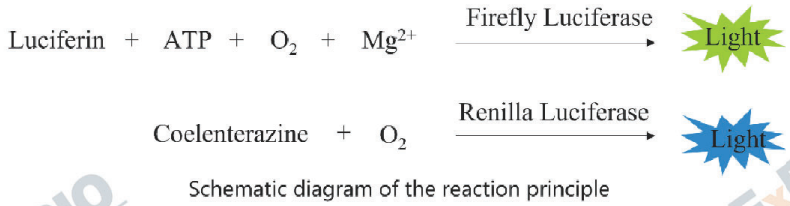
Dual Luciferase Assay System

Introduce:

The dual luciferase reporter gene system is one of the important means to study the regulatory expression of genes. This kit contains high-purity firefly Luciferin and Coelenterazine, which emit bioluminescence of the corresponding wavelength during the reaction, where the firefly fluorescein emits a yellow-green light with a wavelength of 550-570 nm in the presence of firefly luciferase, oxygen, ATP and magnesium ions at the same time, while Coelenterazine can react to emit a blue light with a wavelength of 480 nm in the presence of Renilla luciferase and oxygen.

In the experiment, the expression of firefly Luciferase (Firefly Luciferase) was detected by fluorescein as a substrate, and then the fluorescence signal of firefly luciferase was quenched, and the expression of Renilla luciferase was detected with luciferin as the substrate to achieve dual luciferase reporter gene detection.

This product does not need to lyse the cells with lysate in advance, and then add fluorescence detection reagents, simplifying the experimental process. Luciferase reagent is added directly to the cell culture when used, the cells are lysed, and a firefly luciferase substrate is provided at the same time, which makes this product more suitable for high-throughput detection. Our reagents are suitable for mammalian cell culture media, such as the following types of media containing 1-10% serum: RPMI 1640, DMEM, MEMα and F12, etc.



Schematic diagram of the reaction principle

Materials/Composition

Component	Size
Luciferase Buffer	10 ml
Luciferase Substrate (lyophilized)	1 vial
Stop & Glo Buffer	10 ml
Stop & Glo Substrate (100×)	100 µl

Store conditions

Long-term storage: -20 °C, away from light. Luciferase Buffer, Luciferase Substrate (lyophilized) and Stop & Glo Buffer are stable for 1 year. Stop & Glo Substrate (100×) is stable for 6 months; Shipping conditions: dry ice transport.

Before mixing: Luciferase Buffer is stored at -20 °C, Stop & Glo Buffer can be stored at 4 °C for short periods of time and stored at long-term storage -20 ℃； Luciferase Substrate and Stop & Glo Substrate are stored at -20 °C.

After mixing: unused Luciferase Reagent is stored at -70 °C or below; Repeated freeze-thawing will reduce the efficacy of the reagent. Note that the next time you use it, **do not thaw at temperatures above 25 °C**. The reagent can be melted at 4 °C or room temperature, and this product can also be placed in a 22 °C water bath to melt. Mix well after thawing.

Stop & Glo Reagent is not recommended to be configured in advance, it is configured in the amount needed before the experiment, and after the experiment is completed, the excess unused Stop & Glo Reagent is recommended to be discarded.

Experimental operation

Prepare before the experiment

1. Cell culture plates recommend choosing opaque white cell culture plates to prevent inter-well interference (you can also choose a black cell culture plate according to your needs). This product is suitable for high-throughput detection, and the commonly used cell culture plates are96-well plates and3-84-well plates.
2. When first used, the Luciferase Buffer is melted at 2 ~ 8 °C or room temperature, and this product can also be placed in a 22 °C water bath to melt (the water temperature should not exceed 25 °C), and the Luciferase Substrate can be placed The dry powder is dissolved in the Luciferase Buffer, and the tube containing the Luciferase Substrate can be cleaned using the Luciferase Buffer 2 times, cover the lid, gently invert and mix 3-5 times to fully dissolve the substrate to obtain Luciferase reagent. The assay is stable for several hours at room temperature (Luciferase Reagent loses about 10% of the 8 h at room temperature). Fluorescence intensity).
3. If not for first use, Luciferase Reagent can melt at 4 °C or at room temperature, or it can be melted in a 22 °C water bath. Do not thaw at temperatures above 25 °C. Mix well after thawing.
4. Calculate the volume of Stop & Glo Reagent required for the experiment according to the experimental requirements, and then configure the Stop & Glo Substrate (100 ×) with the Stop

& Glo Buffer in a 1:100 ratio and mix gently upside down to obtain the Stop & Glo Reagent。(The salt concentration in stop & Glo buffer is high, and some precipitation may occur at low temperatures, so wait for it to dissolve completely before mixing the reagents.) We recommend having the Stop & Glo Reagent ready before use. It is not recommended to configure it in advance.

5. The optimal active temperature for firefly luciferase and hydrauline luciferase is room temperature (20–25°C), so it is recommended to equilibrate the reagent and the cell culture plate to be measured to room temperature before use.

Detection steps

1. Remove the mammalian cell culture plate to be measured (96-well or 384-well plate) from the incubator and leave it at room temperature for a period of time to equilibrate its temperature to room temperature 。 The cell culture plates used for the experiment must be compatible with a multifunctional microplate reader with a chemiluminescent module.
2. Glowworm luciferase activity detection: Add a configured Luciferase Reagent in the same volume as the cell culture to be measured and mix well. For example: For 96-well cell culture plates, add 75 µl of Luciferase Reagent to the 75 µl culture. For 384-well cell culture plates, add 20 µl of Luciferase Reagent to the 20 µl culture.
3. Leave at room temperature for 10 min (can be gently mixed on a shaker) to detect the luciferase luminescence intensity of fireflies.
4. Renilla luciferase activity assay: Add a volume of Stop & Glo Reagent equal to the original cell culture to be measured in each well and mix well. For example: For 96-well cell culture plates, add 75 µl Stop & Glo Reagent to the culture under test. For 384-well cell culture plates, add 20 µl stop & Glo Reagent to the culture under test.
5. Leave at room temperature for 10 min (can be gently mixed on a shaker) to measure the luciferase luminescence intensity of the searen.

Notes

1. Stop & Glo Reagent needs to be added to cell culture within 4 h after adding Luciferase Reagent The test is performed in the plate bore.

2. If you have multiple cell culture plates, you need to set up the same control on each multi-well plate to ensure comparable results between the different plates.
3. Detection instruments: It is generally recommended to use a multifunctional microplate reader with a chemiluminescent module. The recommended board reading time is 0.5-1 sec.

Data analysis

It is recommended to set up blank controls, experimental groups, and control groups in each cell culture plate.

Blank control: Untransfected cells, Firefly luciferase and Renilla luciferase detection values are denoted as O_F and O_R, respectively

Experimental group: The transfected cells were treated with experimental compounds, and the test results of Firefly luciferase and Renilla luciferase were recorded as T_F and T_R, respectively.

Control group: Transfected cells were not treated in the group, and the test results of Firefly luciferase and Renilla luciferase were recorded as C_F and C_R respectively.

Experimental Group Ratio = $(T_F - O_F) / (T_R - O_R)$,

Control Group Ratio = $(C_F - O_F) / (C_R - O_R)$

Result (expression multiple) = Experimental Group Ratio / Control Group Ratio. ie

$$\text{Result} = \frac{(T_F - O_F) / (T_R - O_R)}{(C_F - O_F) / (C_R - O_R)}$$