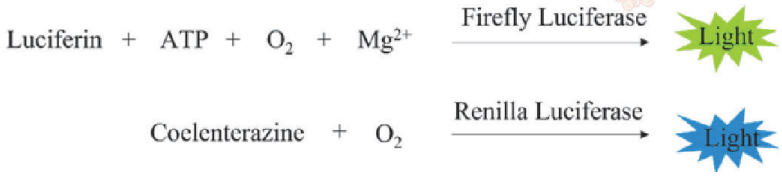
Dual Luciferase Assay System

Introduction

The dual luciferase reporter system is one of the important methods to study the regulation of gene expression. The system use luciferin as a substrate to measure Firefly Luciferase activity, and then use coelenterazine as the substrate to detect Renilla Luciferase activity.

The system is designed for high-throughput screening in mammalian cells grown in 96- or 384-well plates. The Luciferase Reagent can be added directly to cells without washing. This reagent makes cell lysis and serves as a substrate for firefly luciferase. The Stop & Glo Reagent then quenches firefly luminescence and provides a substrate for Renilla luciferase. This system works well in standard mammalian cell growth media with or without serum, offering a versatile and sensitive tool for normalized gene expression analysis.



Components and Storage

Components \ Size	10 mL	Storage
Luciferase Buffer	10 mL	-20°C
Luciferase Substrate (lyophilized)	1 vial	-20°C away from light
Stop & Glo Buffer	10 mL	-20°C
Stop & Glo Substrate (100×)	100 μL	-20°C away from light
Shipping: Dry ice	Shelf life: 6 months	

Protocol

1. Preparation before the experiment

1) It is recommended to choose an opaque white plate for cell culture to prevent interference between wells. Black cell culture plates can also be selected according to your needs. This kit is suitable for high-throughput detection, and the commonly used cell culture plates are 96-well plates and 384-well plates.

2) For first use, thaw the Luciferase Buffer at 2-8°C or room temperature, or in a 22°C water bath (water temperature should not exceed 25°C). mix well after thawing. Transfer 10 mL of Luciferase Buffer to one vial of the Luciferase Substrate to make the Luciferase Reagent. Gently invert several times until the substrate is dissolved thoroughly.

***Note:** The Luciferase Reagent is stable for several hours at room temperature. Luciferase Reagent may have a 10% loss of firefly RLU after 8 h at room temperature.

3) Store unused Luciferase Reagent below -70°C and do not freeze and thaw repeatedly. Thaw unused Luciferase Reagent at 4°C or room temperature, or 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 desired experiment. Then dilute an appropriate volume of Stop & Glo Substrate (100×) 1:100 in Stop & Glo Buffer. Mix well to obtain Stop & Glo Reagent. We recommend preparing Stop & Glo Reagent just before use. Unused Stop & Glo Reagent should be discarded.

***Note:** The salt concentration in the Stop & Glo buffer is high, and some precipitation may occur at low temperatures, so please wait for it to dissolve completely before using.

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

2. Luciferase activity assay

1) Remove the plate containing cells from the incubator and equilibrate it to room temperature for a period of time. Make sure that the plates are compatible with the microplate reader containing the chemiluminescence module.

2) Firefly luciferase activity assay: Add Luciferase Reagent equal to the volume of culture medium in each well. For a 96-well plate, add 75 µL of Luciferase Reagent per well, if 75 µL of medium per well. For a 384-well plate, add 20 µL of Luciferase Reagent per well, if 20 µL of medium per well.

3) Place the plate at room temperature for 10 min, then detect the firefly luminescence.

4) Renilla luciferase activity assay: Add Stop & Glo Reagent equal to the original volume of culture medium in each well. For a 96-well plate, add 75 µL of Stop & Glo Reagent per well, if 75 µL of original medium per well. For a 384-well plate, add 20 µL of Stop & Glo Reagent per well, if 20 µL of original medium per well.

***Note:** Stop & Glo Reagent needs to be added to the plate wells within 4 h of addition of Luciferase Reagent.

5) Place the plate at room temperature for 10 min, then detect the Renilla luminescence.

3. Data analysis

1) It is recommended to set up a negative control, an experimental control group and an experimental group in each plate.

Groups	Treatment	Firefly luciferase and Renilla luciferase values
Negative control	-	O_F and O_R
Experimental control group	co-transfect luciferase plasmids	C_F and C_R
Experimental group	co-transfect luciferase plasmids, treat cell with interested drug	T_F and T_R

2) Refer to the following formula for calculation

Experimental group ratio = $(T_F - O_F) / (T_R - O_R)$,

Experimental control group ratio = $(C_F - O_F) / (C_R - O_R)$.

Result = the experimental group ratio/the experimental control group ratio.

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

Note

1. For your safety and health, please wear lab coats and gloves during the experiment.
2. For research use only. Not to be used in clinical diagnostic or clinical trials.

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