Duo-Lite Luciferase Assay System

DD1205

Vazyme

Instruction for Use

Version 24.1

Vazyme

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01/Product Overview

Duo-Lite Luciferase Assay System is a kind of dual reporter gene assay system featured by high sensitivity, stability and homogeneity. This reagent contains high-purity Firefly luciferin and Coelenterazine. Firefly luciferase is first measured using luciferin as the substrate, and then the catalytic reaction of firefly luciferase is quenched. At the same time, the expression of *Renilla* luciferase is measured using Coelenterazine as the substrate, achieving dual luciferase reporter gene measure.

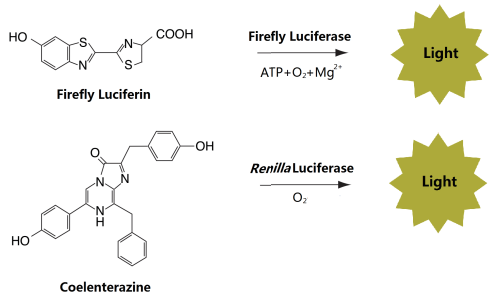

Figure 1. Bioluminescent reactions catalyzed by firefly and *Renilla* luciferases

As shown in Figure 2, Duo-Lite Luciferase Reagent is directly added to the cells in growth medium to lyse the cells and provide firefly luciferase substrate, and the half-life of the generated optical signal can usually reach 2 hours. After that, Duo-Lite Stop & Lite Reagent is added to quench the luminescence of firefly luciferase reaction (the quench efficiency is more than 10,000 times), and the substrate of *Renilla* luciferase is provided, and the generated optical signal could also be read within 2 hours. Therefore, Duo-Lite Luciferase Assay System is more suitable for high-throughput analysis of 96-or 384-well plates. In addition, as an internal reference to correct transfection efficiencies, the *Renilla* luciferase eliminates the influence caused by the difference in the number of cells between wells, transfection efficiencies and cell growth state, which makes the measure result more accurate.

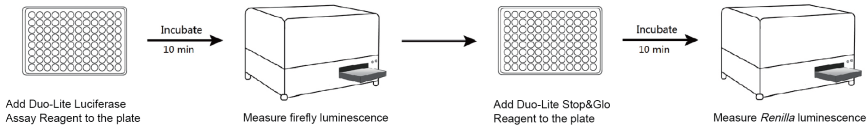

Figure 2. Duo-Lite Luciferase Assay protocol

02/Product components

Component	DD1205-01	DD1205-02
Duo-Lite Luciferase Buffer	10 ml	100 ml
Duo-Lite Luciferase Substrate (lyophilized)	1 vial	1 vial
Duo-Lite Stop & Lite Buffer	10 ml	100 ml
Duo-Lite Stop & Lite Substrate	100 µl	1 ml

03/Scope of application

This product can be used for quantitative analysis of firefly and *Renilla* luciferases expressed in mammalian cells, and is not affected by serum concentration.

04/Storage conditions

Long-term storage: -30 ~ -15℃; Transport conditions: ≤0℃.

Before mixing: Duo-Lite Luciferase Buffer and Duo-Lite Stop & Lite Buffer can be stored at room temperature for a long time to prevent long-term temperature equilibrium when mixing reagents;

Duo-Lite Luciferase Substrate and Duo-Lite Stop & Lite Substrate can be stored at 2 ~ 8℃ for 14 days.

After mixing: Duo-Lite Luciferase Reagent can be stored at room temperature for 1 day (> 80% activity) or at 2 ~ 8℃ for 1 day (> 90% activity);

After 10 cycles of freezing and melting, it can still keep stable. For the reagent that is not used for a long time, to store it at -70℃ is suggested.

Duo Lite Stop & Lite Reagent need to be prepared and used on site.

05/Experimental preparation

Self-provided material

Single/multi-channel pipettor; White/black cell culture plates; Microplate reader with a luminescence detection module.

06/Operation process

Reagent Preparation

1. Melting: Put Duo-Lite Luciferase Buffer at 2 ~ 8℃ or room temperature for melting. The product can also be placed in a 22℃ water bath for melting, but it should be noted that the water temperature should not exceed 25℃.
2. Preparation of Duo-Lite Luciferase Reagent: Add the melted whole bottle of Duo-Lite Luciferase Buffer into Duo-Lite Luciferase Substrate, gently invert and mix evenly for 3 - 5 times to fully dissolve the substrate.
3. Preparation of Duo-Lite Stop & Lite Reagent: Calculate the volume of Duo-Lite Stop & Lite Reagent needed for the experiment. Dilute the Duo-Lite Stop & Lite Substrate to the corresponding volume of Duo-Lite Stop & Lite Buffer at a ratio of 1:100, and gently invert and mix well. Such as: When preparing 5 ml Duo-Lite Stop & Lite Reagent, 50 μl Duo-Lite Stop & Lite Substrate can be added into 5 ml Duo-Lite Stop & Lite Buffer.

▲ Before use, make sure that Duo-Lite Luciferase Buffer and Duo-Lite Stop & Lite Buffer have been balanced to the room temperature. If Duo-Lite Luciferase Reagent is stored at -20℃or -70℃, after the melting, it should gently make the upside down and mixed it well for 3 - 5 times before use.

Assay Procedure

1. Remove multiwell plates containing mammalian cells from the incubator and let it stand at room temperature for 30 minutes to make the temperature of the culture plates balance to room temperature.
2. Measuring firefly luciferase activity: Add Duo-Lite Luciferase Reagent with the same volume as the cell culture to each well and mix evenly. Such as: When using 96-well culture plates, typically 75 µl of reagent is added to cells grown in 75 µl of medium. When using 384-well culture plates,typically 20 µl of reagent is added to cells grown in 20 µl of medium.
3. Leave it at room temperature for 10 minutes to measure the luminescence of firefly luciferase.
4. Measuring *Renilla* luciferase activity: Add Duo-Lite Stop & Lite Reagent with the same volume as the original cell culture to each well and mix evenly. Such as: When using 96-well culture plates, the 75 µl reagent is added to the 75 µl culture. When using 384-well culture plates, the 20 µl reagent is added to the culture.
   ▲ Duo-Lite Stop & Lite Reagent should be added to the plate wells within 4 hours after adding Duo-Lite Luciferase Reagent.
5. The luminescence of *Renilla* Luciferase is measured at room temperature for 10 minutes.
   ▲ The *Renilla* Luciferase luminescence should be measured in the same plate order as that of the firefly.

07/Considerations

1. The cryopreservation can reduce the loss of activity of Duo-Lite Luciferase Reagent. Do not melt the mixed Reagent at a temperature higher than 25℃. It is recommended to keep it in a water bath at 22℃ for a period of time before use to balance it to room temperature. When using, only the required amount of Duo-Lite Stop & Lite Reagent for the experiment should be prepared to ensure the best results. The Duo-Lite Stop & Lite Reagent should be prepared for immediate use.
2. The intensity of luminescence and rate of decay depend on the reaction rate of luciferase. The temperature has a direct effect on the enzyme reaction rate. The optimal temperature for the two luciferases activities is about room temperature (20 ~ 25℃). Therefore, it is crucial to balance the reagent and the culture to room temperature. It is recommended to preserve Duo-Lite Luciferase Buffer and Duo-Lite Stop & Lite Buffer at room temperature. If the temperature of the reagents are lower than room temperature, place them in a 22℃ water bath to balance to room temperature before using.
3. For batch operations, set same control wells on each perforated plate to ensure the comparability of results between plates.
4. Data analysis: For maximal accuracy, the luminescence measurements of both firefly and Renilla luciferases should be background subtracted.

Background Firefly: Nontransfected cells + Duo-lite Luciferase Reagent

Background *Renilla*: Nontransfected cells + Duo-lite Luciferase Reagent +Duo-Lite Stop & Lite Reagent

▲ Sample volumes for background measurements must be the same as experimental sample volumes. And it shall contain the same media/sera combinations as the experimental samples.

According to different experimental purposes, set blank controls in each culture plate, as well as the experimental group and control group:

Blank control: Nontransfected cells deducted with background (i.e., background Firefly and background *Renilla*)

Experimental group: Transfected cells treated with an experimental compound (i.e., experimental group Firefly and experimental group *Renilla*)

Control group: Transfected cells were not treated to standardize the results (i.e. control group Firefly and control group *Renilla*).

$$\text{Final results} = \frac{\begin{array}{c}\text{(Experimental group Firefly - background Firefly)/}\\ \text{(Experimental group } \textit{Renilla} \text{ - background } \textit{Renilla}\text{)}\end{array}}{\begin{array}{c}\text{(Control group Firefly - background Firefly)/}\\ \text{(Control group } \textit{Renilla} \text{ - background } \textit{Renilla}\text{)}\end{array}}$$

5. This product is only for scientific research use, shall not be used for clinical medical diagnosis and other irrational purposes.

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