TOOLShinning Dual Luciferase Reporter Assay Kit

For detecting gene regulation

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Introduction

The TOOLShinning Dual Luciferase Reporter Assay Kit detects gene regulation by transfecting cells with a reporter plasmid and measuring the fluorescence intensity of luciferin substrate to reflect the level of luciferase expression. To achieve dual luciferase reporter gene detection, firefly luciferase is detected using luciferin as a substrate. Moreover, Renilla luciferase is detected using coelenterazine as a substrate and inhibits the activity of firefly luciferase. The aforementioned assay kit can detect the expression of luciferase regulated by gene elements with high sensitivity and efficiency. Usually, the transcriptional regulatory element is cloned upstream of firefly luciferase or the 3'-UTR regulatory region is cloned downstream of firefly luciferase. The transfected cells are induced by the corresponding stimulator and lysed to determine the luciferase activity. The stimulation-inducing effect of the regulatory elements is evaluated using luciferase activity. Renilla luciferase acts as an internal reference for correcting the transfection efficiency to eliminate differences in cell numbers and transfection efficiency between the wells. Firefly luciferase catalyzes the emission of luciferin at 560 nm, and Renilla luciferase catalyzes the emission of coelenterazine at 465 nm.

Assay preparation

1. First-time users should pour the luciferase buffer into a dark brown bottle containing lyophilized luciferase substrate. Mix thoroughly, use according to the experiment requirements, and keep away from light at −70 °C.
2. Mix 5× lysis buffer and ddH_2O at a ratio of 1:4 before use, and place the mixture on ice for use.
3. The Renilla substrate is dissolved in ethanol. In the first instance, centrifuge briefly. Carefully measure the volume of the solution in the tube. If the volume of the liquid is significantly reduced, add ethanol to compensate for the volume.
4. Place the Renilla substrate on ice for use. Calculate the actual usage, mix an appropriate amount of stop–go buffer and Renilla substrate at a ratio of 50:1, and keep the mixture away from light at room temperature.
5. The enzymatic reaction is sensitive to temperature. The cell lysis and detection substrate solutions should be equilibrated to room temperature before a sample is tested.
6. Detection instrument: Instruments capable of detecting chemiluminescence are suitable for this kit. However, for the same sample, the value of the background signal and measurements of various detection instruments may be different. Therefore, the background signal of a fluorescent substrate must be detected in a preliminary experiment. The values provided by different instruments should not be compared horizontally. If a full-spectrum microplate reader is used for detection, use of an opaque microplate is recommended, and a certain interval should be ensured between the detection wells.

Kit Contents

Contents	TTC-DLA01 (100 rxn)
5× lysis buffer	10 mL
Luciferase buffer	10 mL
Luciferase substrate (lyophilized)	One vial
Stop–go buffer	10 mL
Renilla substrate	200 μL

Storage

The TOOLShinning Dual Luciferase Reporter Assay Kit should be stored at −20 ℃. Dissolved and dispensed luciferase substrate can be stored at −70 ℃ over a long period or at −20 ℃ for less than 1 month.

Protocol

1. Cell lysis

Discard the cell culture medium, and wash the cells twice with PBS. Add an appropriate amount of 1× lysis buffer as per the recommendations in the following table. Stand the mixture or shake it for 5 min at room temperature, pipette the mixture, and transfer the cell lysate into a 1.5-mL centrifuge tube. Centrifuge the cell lysate for 2 min at 12 000 × g at room temperature, and collect the supernatant formed for subsequent testing.

Cell Culture Plate	1 × Cell Lysis Buffer
6-well	500 μl
12-well	200 μl
24-well	100 μl
48-well	50 μl
96-well	20 μl

If the expression level of luciferase is too low, the amount of lysis buffer can be appropriately reduced to increase the protein concentration.

2. Firefly luciferase activity detection

Add 100 μL of luciferase substrate that has been equilibrated to room temperature to the detection tube or microplate. Carefully pipette 20 μL of the cell lysate into the test tube or plate. Immediately after the rapid mixing, detect the firefly luciferase reporter gene activity by using a luminometer detector or full-spectrum microplate reader.

3. Renilla luciferase activity detection

Add 100 μL of freshly prepared Renilla substrate solution to the aforementioned reaction solution. Immediately after the rapid mixing detect the Renilla luciferase reporter gene activity by using a luminometer detector or full-spectrum microplate reader.

Important notes

1. The optimum lysis time may vary for different cell lines. An initial lysis time of 5 min is recommended, and the lysis time can be extended to 10 min for complete lysis. After lysis is completed, do not pipette the cells for a long time to prevent the production of large amounts of foam, which may affect enzyme activity.
2. If the expression level of luciferase is too low, the amount of lysis buffer can be appropriately reduced to increase the protein concentration. Increase the number of duplicate wells to reduce the differences between the pores caused by low concentration expression to ensure the reliability of the results.
3. In general, the addition of the stop–go buffer can inhibit more than 99% of the activity of firefly luciferase; however, trace activity may remain. Therefore, controlling the RLU value of the expression of Renilla luciferase at a level comparable to or marginally higher than that of firefly luciferase during transfection is recommended.
4. The fluorescence intensity becomes stable within approximately 1 minute after the lysate comes into contact with the substrate. When using a single-tube chemiluminometer, to obtain the best results, the time interval between the mixing of various samples and substrates and detection with the machine should be as consistent as possible. When using a full-spectrum microplate reader, the cell lysate should first be added to the well. Subsequently, the detection substrate should be added to the well and tested with the instrument as soon as possible. The measurement time can be set between 1 and 10 sec according to the intensity of the fluorescence. The fluorescence reading of the sample and the intensity of the background signal increase with the detection time.