Dual Luciferase Reporter Assay Kit

Catalog Number: KC134-100

Storage Condition: -20℃

Expiration date: Store at -20ºC, valid for one year

product description

The Dual Luciferase Reporter Assay Kit is used to detect gene regulation by measuring the fluorescence intensity of Luciferin substrate to reflect the expression level of Luciferase after transfecting cells with reporter plasmid. Firefly luciferase is detected with Luciferin as a substrate, Renilla luciferase is detected with Coelenterazine as a substrate, and the catalytic reaction of Firefly luciferase is inhibited , To achieve dual luciferase reporter gene detection. The Dual Luciferase Reporter Assay Kit can sensitively and efficiently detect the expression of Luciferase regulated by genetic elements. Generally, transcriptional regulatory elements are cloned upstream of Firefly luciferase, or the 3'UTR regulatory region is cloned downstream of Firefly luciferase. After transfection, the corresponding Stimulus induction, lysis of cells and determination of luciferase activity. The luciferase activity was used to evaluate the strength of the regulatory element induced by the stimulus. ORenilla luciferase was used as an internal reference to correct the transfection efficiency to eliminate the difference in cell number and transfection efficiency between wells. luciferase catalyzes Coelenterazine emission at 465 nm

Product components

components	KC134-100 100 rxn (100 pl/rxn)
5 x Cell Lysis Buffer	10 ml
Reaction Buffer II (Luciferase) Luciferase Substrate	10 ml
(Lyophilized) Stop & Reaction Buffer	1 vial
Renilla Substrate	10 ml
	200 pl

Storage Conditions

Store the kit at -20 ° C;

Luciferase Substrate after dissolving and packing can be stored at -70 ° C for a long time, or -20 "C for a short period of less than one month;

Experiment preparation and guide

Bring your own materials

PBS; pipette or row gun; microplate (recommended black); Luminometer luminometer or full wavelength microplate reader.

user's guidance

• When using for the first time, pour Reaction Buffer II (Luciferase) into Luciferase Substrate brown light-shielding bottle containing lyophilized substrate, mix thoroughly and dispense according to the requirements of use and store at T-70 ° C in the dark;

• 5 x Cell Lysis Buffer and ddbh in a 1: 4 ratio before each use. Mix well and keep on ice for later use;

• Renilla Substrate is dissolved in absolute ethanol. Please centrifuge briefly for the first time, and carefully measure the solution volume in the tube. If the liquid volume is significantly reduced, add absolute ethanol to make up the volume and save;

• When using Renilla Substrate, temporarily store it on ice, calculate the actual amount of use, mix an appropriate amount of Stop & Reaction Buffer and Renilla Substrate at a ratio of 50: 1, and keep the room temperature away from light;

• The enzymatic reaction is more sensitive to temperature, so the cell lysate and the test substrate solution should be equilibrated to room temperature before loading and detection;

• Selection of detection instruments: Instruments that can detect chemiluminescence are suitable for the detection of this kit, but the background signal value and measurement value of different detection instruments may be different for the same sample, so it is necessary to add only fluorescence in the test The background signal of the substrate, and for the detection of the same sample, the values of different instruments cannot be compared laterally. If using a full-wavelength microplate reader for detection, in order to prevent interference between the wells, it is recommended to use an opaque plate with a certain interval between the wells.

experiment process

Cell lysis

Aspirate the cell culture medium and wash it twice with PBS. Add the appropriate amount of 1 x Cell Lysis Buffer as shown in the table below. Leave at room temperature or shake and lyse for 5 min. Pipette and draw the cell lysate into a 1.5 ml centrifuge tube, 12000 g at room temperature. Centrifuge for 2 min and take the supernatant for subsequent detection.

Cell Culture Plate	1 * Cell Lysis Buffer
6-well	500 yl
12-well	200 pl
24-well	100 |I
48-well	50 pl
96-well	20 pl

▲ If the expression level of luciferase is too low, you can reduce the amount of lysate to increase the protein concentration.

2.Firefly luciferase reaction detection

Add 100 I Luciferase Substrate equilibrated to room temperature to the test tube or microtiter plate. Carefully pipette the 20 I cell lysate supernatant into the test tube or microtiter plate well. Mix quickly and immediately in a Luminometer. Or check the activity of the Firefly luciferase reporter gene in a microplate reader.

3. Renilla luciferase reaction detection

Add 100 pl of freshly prepared Renilla substrate working solution to the above reaction solution, and immediately mix the Renilla luciferase reporter gene activity in a fluorescence detector or microplate reader immediately after mixing.

Precautions

1. For different cell lines, the optimal lysis time may be different. It is recommended to start from 5 minutes and can be extended to 10 minutes to obtain a more sufficient lysis effect. It is not advisable to blow the cells for too long after the lysis is complete, in order to prevent the production of large amounts of vesicles that affect the enzyme activity.

2. If the amount of luciferase expression in the system is low, the amount of lysate can be appropriately reduced to increase the protein concentration. At the same time, the number of duplicate wells should be increased to reduce the difference between wells caused by low concentration expression to ensure the reliability of the results.

3.Usually, Stop & Reaction Buffer is added to inhibit the previous step Firefly luciferase, which can reach 99% or more. However, trace activity may remain. Therefore, it is recommended to control the expression of Renilla luciferase at its RLU reading and Firefly during transfection. Luciferase is equivalent or slightly higher.

4. The fluorescence intensity is relatively stable within about 1 minute after the lysate is in contact with the substrate. In order to obtain the best detection results, when using a single tube chemiluminometer, the time interval between the mixing of different samples and substrates and the detection on the machine should be Try to be consistent; when using a full-wavelength microplate reader, you should first add the cell lysate to the well, then add the detection substrate uniformly and test it on the machine as soon as possible. The measurement time can be set within the range of 1-10 s according to the intensity of the fluorescence value. Increasing the detection time will increase the fluorescence readings of the sample and the background at the same time.