

## Dual Luciferase Reporter Assay Kit

Cat# NB-54-0020 size 100rxns

### Product Description

The Dual Luciferase Reporter Assay Kit is used to detect 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 with Luciferin as a substrate, and *Renilla* luciferase is detected with coelenterazine as a substrate, while inhibiting the activity of Firefly luciferase. Dual Luciferase Reporter Assay Kit can detect the expression of Luciferase regulated by gene elements sensitively and efficiently. 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 corresponding stimulator and lysed to determine the luciferase activity. The stimulatory-inducing effect of the regulatory elements is evaluated by luciferase activity. *Renilla* luciferase acts as an internal reference for correcting transfection efficiency to eliminate differences in cell number and transfection efficiency between wells. Firefly luciferase catalyzes the emission of Luciferin at 560 nm, and *Renilla* luciferase catalyzes the emission of coelenterazine at 465 nm.

### Components

Components	NB-54-0020 (100 rxns)
5 × Cell Lysis Buffer	10 ml
Reaction Buffer II (Luciferase)	10 ml
Luciferase Substrate (Lyophilized)	1 vial
Stop & Reaction Buffer	10 ml
Renilla Substrate	200 µl

### Storage

Store at -30 ~ -15°C and transport at ≤0°C.

Dissolved and dispensed Reagent: long-term storage at -85 ~ -65°C, or short-term storage at -30 ~ -15°C for no more than one month;

Stop & Reaction Buffer: long-term storage at 2 ~ 8°C.

### Applications

It is applicable for the detection of cell gene regulation.

### Self-prepared Materials

PBS, Pipette, Microplate plate (black), Full wavelength microplate reader or luminometer.

### Notes

For research use only. Not for use in diagnostic procedures.

1. When used for the first time, the Reaction Buffer II (Luciferase) should be poured into a brown dark bottle containing lyophilized Luciferase substrate. Mix thoroughly and dispense according to the needs of use and protect from light at -70°C.
2. Mix 5 × Cell Lysis Buffer and ddH<sub>2</sub>O in a ratio of 1:4 before use, and place on ice for use.
3. Dissolve *Renilla* Substrate in absolute ethanol. Please centrifuge briefly for the first use and carefully measure the volume of the solution in the tube. If the liquid volume is found to be significantly reduced, please add absolute ethanol to make up the volume before saving.
4. Place *Renilla* Substrate on ice during use. Calculate the usage amount, mix an appropriate amount of Stop & Reaction Buffer and *Renilla* Substrate at a ratio of 50:1. Store at room temperature and protect from light for later use.
5. The enzymatic reaction is sensitive to temperature, so the cell lysis solution and the detection substrate solution should be equilibrated to room temperature before the sample is applied for testing.

6. Detection instrument selection: Instruments capable of detecting chemiluminescence are suitable for this kit. But for the same sample, the value of background signal and measurement of different detection instruments may be different, so it is necessary to detect the background signal of fluorescent substrate in the pre-experiment. The values of the different instruments should not be compared horizontally. If a full-spectrum microplate reader is used for detection, it is recommended to use an opaque microplate and guarantee a certain interval between the detection wells.
7. The optimum lysis time may vary for different cell lines. It is recommended to start from 5 min, and the lysis time can be extended to 10 min for a complete lysis. After the lysis is completed, please do not pipet the cells for a long time to prevent the production of large amounts of foam, which may affect the enzyme activity.
8. If the expression level of luciferase is too low, the amount of Cell Lysis Buffer can be appropriately reduced to increase the protein concentration. And increase the number of duplicate wells to reduce the difference between wells caused by low concentration expression to ensure the reliability of the results.
9. In general, the addition of the Stop & Reaction Buffer can inhibit the more than 99% of the activity of Firefly luciferase, however, there may be trace activity left. Therefore, it is recommended to control the RLU value of expression of *Renilla* luciferase at a level comparable to or slightly higher than that of Firefly luciferase during transfection.
10. The fluorescence intensity is stable within about one minute after the lysate is in contact with the substrate. When using a single-tube chemiluminometer, to obtain the best results, the time interval between the mixing of different samples and substrates and the detection on the machine should be as consistent as possible. When using a full-spectrum microplate reader, the cell lysate should be added in the well first, then the detection substrate should be added and tested on the instrument as soon as possible. The measurement time can be set between 1 - 10 sec according to the intensity of the fluorescence value. Increasing the detection time will increase the fluorescence reading of the sample and the background at the same time.

## Experiment Process

### 1. Cell lysis

Discard the cell culture medium and wash the cells twice with PBS. Add the appropriate amount of 1 × Cell Lysis Buffer as recommended in the table below. Stand still or shake for 5 min at room temperature, pipette up and down and transfer the cell lysate into a 1.5 ml centrifuge tube. Centrifuge at 11,200 rpm (12,000 × g) for 2 min at room temperature, and collect the supernatant 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 Cell Lysis Buffer can be appropriately reduced to increase the protein concentration.

### 2. Firefly luciferase activity detection

Add 100 μl of Luciferase Substrate (which has been equilibrated to room temperature) to the detection tube or microplate. Carefully pipet 20 μl of the cell lysate into the test tube or the plate. After mixing quickly, immediately detect the activity of the Firefly luciferase reporter gene in a luminometer or a microplate reader.

### 3. *Renilla* luciferase activity detection

Add 100 μl of freshly prepared *Renilla* Substrate solution to the above reaction solution, After mixing quickly, immediately detect the activity of the *Renilla* luciferase reporter gene in a luminometer or a microplate reader.