Product Information

Firefly & Renilla Dual Luciferase Assay Kit

Catalog Number: F6075

Product Size: 100 T, 1000 T

Contents:

Component	100 T	1000 T
A. 5× Passive Luciferase Lysis Buffer	10 mL	100 mL
B. Firefly Luciferase Assay Buffer	10 mL	100 mL
C. D-Luciferin	2 mg	20 mg
D. Renilla Luciferase Assay Buffer	10 mL	100 mL
E. 50 × Coelenterazine	200 µL	2 mL

Storage

Store at -80 ℃. Component A, B and D is stable for at least 3 months at -20 ℃ and 6 months at -80 ℃. Component C and E is stable for at least 6 months at -20 ℃ in dark. Firefly luciferase working solution (B + C) is stable for one month at -20 ℃ and 3 months at -80 ℃, and do not freeze and thaw repeatedly, it is recommended to pack in small batches. Renilla Luciferase Assay solution(D + E) should be freshly prepared and used on the same day.

Description

Firefly & Renilla Dual Luciferase Assay Kit provides an effective means for double reporter gene detection. In DLR detection, the activity of Firefly luciferase and Renilla luciferase can be detected in a single sample in turn. First, use luciferin as substrate to detect Firefly luciferase, and then use coelenterazine as substrate to detect Renilla luciferase, and when luciferase substrate is added subsequently, substances that inhibit luciferase catalytic luciferin luminescence are added at the same time, so that the subsequent detection only detects luciferase activity of Renilla luciferase and realizes the detection of double luciferase reporter gene. Luciferase and its substrate, a bioluminescent system, can detect gene expression very sensitively and efficiently. Usually, the transcriptional regulatory element or 5 'promoter region of the gene of interest is cloned upstream of luciferase, or the 3' - UTR region is cloned downstream of luciferase, etc. to construct a reporter gene plasmid, and then transfect into the cells. After the cells are treated with appropriate drugs, the cells are split to determine the luciferase activity. Through the activity of luciferase, we can judge the transcriptional regulation of target gene by drug treatment. Renilla luciferase was used as an internal parameter of transfection efficiency to eliminate the difference of cell number and transfection efficiency.

Firefly luciferase, a monomeric 62,000 Dalton protein, catalyzes ATP-dependent D-luciferin oxidation to oxyluciferin to produce light. Renilla luciferase has been used as a reporter gene for studying gene regulation and function in vitro and in vivo. It commonly is used in multiplex transcriptional reporter assays or as a normalizing transfection control for firefly luciferase assays. Renilla luciferase, a monomeric 36,000

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Dalton protein, catalyzes coelenterazine oxidation by oxygen to produce light. This kit has the characteristics of rapid detection, high sensitivity, wide detection range and no interference of endogenous activity.

Protocol

1. Cell lysis

1.1 Remove culture medium from cells transferred with reporter gene and wash twice with PBS (adherent cells can wash directly, and suspension cells need centrifugation to be collected). Add 1 × Lysis Buffer as follows (dilute component A with sterile water for 4 times), and then place the culture plate on the micro vibrator for 15 min at room temperature to fully lyse cells.

Culture plate	96-well plate	48-well plate	24-well plate	12-well plate	6-well plate
Lysis Buffer	30 μL	60 μL	120 μL	250 μL	500 μL

Note: Cells after lysis can be stored at room temperature for 6 h and at -70 ℃ for a long time (Pyrolysis products cannot be repeatedly frozen and thawed). If the expression level of luciferase is low, you can try to use less lysate, for example, the amount of 6-well plate can be 100 μL per well.

1.2 Pyrolysis products were centrifuged at 10000-15000 rpm for 3-5 min. After centrifugation, the supernatant was transferred into a new EP tube for subsequent detection.

Note: Luciferase can be detection immediately after cell lysis, or it can be cryopreserved first and determined later. The cryopreserved samples need to be thawed and measured after reaching room temperature.

2. Preparation of working solution

2.1 0.2 mg/mL firefly luciferase detection solution was prepared by fully dissolving component C with component B.

Note: Working solution of firefly luciferase cannot be frozen and thawed repeatedly. If dosage for single experiment is small, it is recommended to pack it into small size according to the amount of single experiment.

2.2 Dilute component E into 1 × coelenterazine working solution with component D(eg.add 1 μL component E into 49 μL component D).

Note: 1 × coelenterazine working fluid should be used immediately.

3. **Chemiluminescence value detection**

3.1 Restore the prepared firefly fluorescein detection solution and 1 × coelenterazine working solution to room temperature.

3.2 Turn on the chemiluminescent instrument or the multifunctional enzyme labeling instrument with the function of detecting chemiluminescent according to the operation instructions of the instrument. Set the measurement interval to 2 seconds and the measurement time to 10 seconds.

3.3 Take 20-100 μL of sample for measuring(if the sample quantity is enough, please add 100 μL; if the sample quantity is insufficient, the dosage can be appropriately reduced, but the usage of the same batch of samples should be consistent).

3.4 Add 100 μL firefly luciferase detection reagent, beat well with pipetting gun or other appropriate methods, and then determine RLU (relative light unit). The lysate of reporter gene cells was used as blank control.

Note: Since luminescence is instantaneous, it is recommended to detection the luminescence value immediately after adding firefly luciferase detection reagent.

3.5 After the determination of luciferase of firefly, add 100 μL of renilla luciferase detection working solution, mix thoroughly, with pipetting gun or other appropriate methods, and then measure RLU (relative light unit).

3.6 Taking renilla luciferase as internal reference, then the RLU value determined by firefly luciferase was divided by the RLU value determined by renilla luciferase According to the ratio obtained, the activation degree of target reporter gene was compared among different samples. If firefly luciferase is used

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as the internal reference, a similar calculation can be carried out.

Notes

1. In order to obtain the best measurement effect, the time from the mixture of sample and reagent to the time before determination should be controlled alike as much as possible when using the single tube chemiluminescent apparatus; when using the multi-functional fluorescent enzyme reader with chemiluminescent function, it is advisable to add all the samples first, and then add the fluorescent luciferase detection reagent uniformly.

2. Because the temperature has an effect on the enzyme reaction, the sample and reagent should be determined after reaching the room temperature.

3. For your safety and health, please wear lab coats and disposable gloves.

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