Single Firefly Luciferase Reporter Gene Assay Kit II

Introduction

Firefly luciferase is a protein with a molecular weight of approximately 61 kD, which catalyzes the oxidation of the substrate luciferin to oxyluciferin in the presence of ATP, oxygen, and magnesium ions. During the oxidation of luciferin, a bioluminescence with a wavelength of about 560 nm is emitted, which can be measured by a luminometer or a liquid flash meter. The bioluminescence system of luciferin and luciferase allows for very sensitive and efficient detection of gene expression. 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, transfect the cells, and treat the cells with appropriate drugs, and finally measure the luciferase activity in the cell lysate and judge the transcriptional regulation effect of the drug treatment on the target gene by the level of luciferase activity.

Single Firefly Luciferase Reporter Gene Assay Kit II is a high-sensitivity, high-signal robust, one-step assay for the direct determination of intracellular firefly luciferase activity by chemiluminescence without the need to wash or collect cells.

This product is a different packaging version of the Firefly Luciferase Reporter Gene Assay Kit (K2236), and the detection results of both are identical. K2236 is a ready-to-use reagent, the advantage is that it can be used directly without preparation, and the disadvantage is that it needs to be stored at -80ºC, and the detection effect will gradually decrease after a long storage time at -20ºC. This product is a lyophilized powder version of K2236 that needs to be fully dissolved before use, but it can be stored at -20ºC stably.

Components and Storage

Components	K2246-100 Tests	K2246-1000 Tests	Storage
Single Firefly Luciferase Assay Substrate	1 vial	10 vials	-20°C away from light
Single Firefly Luciferase Assay Buffer	10 mL	10×10 mL	-20°C
Shipping: Dry ice	Shelf life: 1 year		

Protocol

1. **Cell seeding:** Cells are first seeded using a well plate suitable for chemiluminescence detection, such as a white or black opaque plate. For 96-well plates, seed 100 μL of cells per well (25 μL of cells per well for 384-well plates). The seeding volume of other plates can be adjusted according to the size of the plate. At the

same time, the wells of the culture medium without cells were set up as a negative control, and the cells are cultured and transfected according to the conventional methods. If needed, drugs can be added to treat the cells.

2. **Prepare assay reagents:** Thaw frozen Single Firefly Luciferase Assay Substrate and Single Firefly Luciferase Assay Buffer. Dissolve a vial of Single Firefly Luciferase Assay Substrate with 10 mL Single Firefly Luciferase Assay Buffer to make the Single Firefly Luciferase Assay Reagent.

***Note:** A small amount of lyophilized powdered Single Firefly Luciferase Assay Substrate may adhere to the cap and bottle mouth, you can pick up the bottle and gently tap the table with the bottom of the bottle before unscrewing the cap to make the powder fall to the bottom of the bottle as much as possible, and then gently unscrew the cap, and be careful not to lose the lyophilized powder.

3. **Firefly luciferase assay:**

    1) Equilibrate the cell culture plate at room temperature for 10 min (usually no more than 30 min).

    2) Add Single Firefly Luciferase Assay Reagent equal to the volume of culture medium in each well. For a 96-well plate, add 100 μL of Single Firefly Luciferase Assay Reagent per well, if 100 μL of medium per well.

    3) Incubate at room temperature (about 25°C) for 5 min to allow the cells to fully lyse and the luminescence signal to stabilize.

    4) Perform Chemiluminescence detection using a multi-mode microplate reader with a chemiluminescence detection function. Please set the corresponding parameters according to the requirements of the instrument, and the detection time of each well is generally 0.25-1 s or longer, which needs to be adjusted appropriately according to the detection sensitivity of the instrument.

Note

1. Before using this kit, the substrate lyophilized powder needs to be dissolved to prepare a homogeneous solution. If you would like to use a ready-to-use solution, you can choose the ready-to-use form of this product (Cat. No. K2236).

2. Since the activity of luciferase is sensitive to temperature, both cells and detection reagents need to be equilibrated at room temperature before the reaction. The detection reagent can be thawed and mixed at room temperature or in a water bath no more than 25°C.

3. Although the reagent has been tested to freeze and thaw 5 times without significant effect on its detection effect, in order to ensure the stability of this product and achieve good use effect, the method of appropriate storage in the dark after the first thaw can be taken to avoid repeated freeze-thaw and long-term exposure to room temperature. Repeated freeze-thaw processes may cause a small amount of precipitation in the detection reagent, so it is advisable to equilibrate to room temperature and dissolve as much as possible. If there is still residual insoluble substance, it can be used after centrifugation and removal, and it will not affect

the subsequent detection effect after testing.

4. Use a white or black 96-well plate or a 384-well plate that is suitable for cell culture. If you use a plain clear 96-well plate or a 384-well plate, adjacent wells will interfere with each other.

5. High solvent levels of the drug to be tested may interfere with the luciferase reaction, which can affect the chemiluminescence signal. Solvent interference can be excluded by setting up a control well of a cell culture medium containing solvent. After testing, the DMSO content in the final reaction system within 2% will not affect the reaction.

6. In order to avoid errors caused by differences in cell transfection efficiency, if necessary, Renilla luciferase reporter plasmid can be transfected simultaneously as an internal control, and the Dual Luciferase Assay System (K1136) can be used for detection.

7. For your safety and health, please wear a lab coat and disposable gloves for operation.

8. This product is for scientific research purposes only.

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