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Datasheet

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Dual Luciferase Reporter Assay Kit

Cat# ER1009 – 100 rxn

Storage at -20 ℃

INTRODUCTION

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.

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.

CONTENTS

No	Component	**ER1009 – 100 rxn**
EA	5X Cell Lysis Buffer	10 ml
EB	Luciferase Reaction Buffer	10 ml
EC	Luciferase Substrate (Lyophilized)	1 Vial
ED	Stop & Reaction Buffer	10 ml
EE	Renilla Substrate	200 µl

STORAGE

Storage at -20 ℃. Dissolved and dispensed Luciferase Substrate can be stored at -70℃ for long term, or at -20℃ for less than one month

ADDITIONAL MATERIAL REQUIRED

1. PBS, pipette or multichannel pipette
2. Immunoassay microplates (black is preferred)
3. Luminometer detector or full-spectrum microplate reader.

PROTOCOL

NOTE:

1. When used for the first time, the Luciferase Reaction Buffer should be poured into a brown dark bottle containing lyophilized Luciferase substrate. Mix thoroughly and dispense according to the needs of use and keep away from light at -70℃.
2. Renilla Substrate is dissolved in ethanol. For the first time, please centrifuge briefly. Carefully measure the volume of the solution in the tube. If the volume of the liquid is significantly reduced, please add ethanol to make up for the volume.
3. Place Renilla Substrate on ice for use. Calculate the actual usage, mix the appropriate amount of Stop & Reaction Buffer and Renilla Substrate in a ratio of 50 : 1, and keep it from light at room temperature.

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.[a] Stand still or shake for 5 min[b] at room temperature, pipette up and down and transfer the cell lysate into a 1.5 ml centrifuge tube. Centrifuge for 2 min, 12000 × g at room temperature, and collect the supernatant for subsequent testing.

Culture Plate	1X Cell Lysis Buffer
6 Well	500µl
12 Well	200µl
24 Well	100µl
48 Well	50µl
96 Well	20µl

a. If the expression level of luciferase is too low, the amount of Cell Lysis Buffer can be appropriately reduced to increase the protein concentration.
b. 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.

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 rapidly, detect the Firefly luciferase reporter gene activity by a luminometer detector or a full-spectrum microplate reader Immediately.

3. Renilla luciferase activity detection

Add 100 µl of freshly prepared Renilla Substrate solution to the above reaction solution, and immediately after mixing rapidly, detect the renilla luciferase reporter gene activity by a luminometer detector or a full-spectrum microplate reader.

ADDITIONAL INFORMATION

1. 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.
2. 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.

PRODUCT USE LIMITATION

These products are intended for research use only.