（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-F071

Specification：48T/96T

Measuring instrument：Chemiluminescence immunoassay analyzer

Elabscience® Dual Luciferase Reporter Gene Luminescence Assay Kit (Flash Type)

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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Intended use

This kit can be used to detec the expression levels of firefly luciferase reporter gene and sea kidney luciferase reporter gene in low-expression sample system.

Detection principle

Dual luciferase reporter gene uses luciferin to detect the luminescence reaction produced by luciferin substrate. The detection system includes two parts: firefly luciferase reporter gene and renilla luciferase reporter gene. As an internal parameter of transfection, the renilla luciferase can normalize the experimental data.

The reaction principle of this test box is that in the presence of oxygen, ATP and magnesium ions at the same time, firefly luciferase and coelentin are catalyzed and oxidized successively by firefly luciferase and renilla luciferase in the samples to produce different fluorescence signals, and the expression levels of firefly luciferase and renilla luciferase in the samples can be detected by chemiluminescence instrument.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Lysis Buffer	12.5 mL × 1 vial	25 mL × 1 vial	-20℃, 12 months
Reagent 2	Firefly Luciferase Buffer Solution	7 mL × 1 vial	14 mL × 1 vial	-20℃, 12 months
Reagent 3	Firefly Luciferase Substrate	Power × 2 vials	Power × 4 vials	-20℃, 12 months shading light
Reagent 4	Renilla Luciferase Buffer Solution	6 mL × 1 vial	12 mL × 1 vial	-20℃, 12 months
Reagent 5	Renilla Luciferase Substrate	0.12 mL × 1 vial	0.24 mL × 1 vial	-20℃, 12 months shading light
	Black Clear-bottom Culture Plate	96 wells		No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Chemiluminescence immunoassay analyzer or multifunctional microplate reader (with the function of detecting luminescence)

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of firefly luciferase substrate working solution: Dissolve one vial of firefly luciferase substrate with 3 mL of firefly luciferase buffer solution, mix well to dissolve. Aliquoted storage at -20°C for 1 month protected from light.

③ The preparation of renilla luciferase substrate working solution: Before testing, please prepare sufficient renilla luciferase substrate working solution according to the test wells. For example, prepare 500 μL of renilla luciferase substrate working solution (mix well 5 μL of renilla luciferase substrate and 495 μL of renilla luciferase buffer solution). The renilla luciferase substrate working solution should be prepared on spot.

Sample preparation

Sample preparation

Cell samples:

① The cells were inoculated and designed according to the following groups:

Blank group: The cells without transfection treatment;

Control group: The cells were transfected with plasmids without drug stimulation.

Experiment group: The cells were transfected with plasmids and stimulated with drugs according to experimental design.

② Cell lysis;

Adherent cells: Removing culture, according to the table below and add corresponding volume of lysis buffer to lyse the cells for 15-30 min. Centrifuge at 15000×g for 5 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection (If the cell sample is not obviously cloudy, centrifugation can be omitted).

Suspension cells: Centrifuge at 500×g for 5 min at 4℃ to remove supernatant. According to the table below and add corresponding volume of lysis buffer to lyse the cells for 15-30 min. Centrifuge at 15000×g for 5 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection (If the cell sample is not obviously cloudy, centrifugation can be omitted).

	6-well plate	12-well plate	24-well plate	48-well plate	96-well plate
Lysis buffer (μL)	500	300	200	150	100

The key points of the assay

① The firefly luciferase substrate working solution should be aliquoted storage at -20°C, and avoid repeated freeze/thaw cycles is advised.

② It is recommended that the number of samples for an experiment be controlled within 5 samples.

Operating steps

① Sample well: add 20 μL of samples into the sample wells. Add 100 μL of firefly luciferase substrate working solution into sample wells, and mix fully with chemiluminescence immunoassay analyzer. Measure the luminescence values of each well, as F_1.

② Sample well: add 100 μL of renilla luciferase substrate working solution into the sample wells, and mix fully with chemiluminescence immunoassay analyzer. Measure the luminescence values of each well, as F_2.

Calculation

Relative Light Units (RLUs) formula:

$$RLUs = \frac{\Delta F1}{\Delta F2}$$

[Note]

ΔF_1: The F_1 values of experiment group - The F_1 values of blank group.

ΔF_2: The F_2 values of experiment group - The F_2 values of blank group.

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.