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

Catalog No：E-BC-F072

Specification：48T/96T

Measuring instrument：Chemiluminescence immunoassay analyzer

Elabscience® Firefly 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 detect the detection of low expression level of firefly luciferase.

Detection principle

Firefly Luciferin reporter gene detection is a reporting system that uses luciferin as a substrate to detect the activity of Firefly luciferase.

The detection principle of this kit: In the presence of oxygen, ATP and magnesium ions at the same time, firefly luciferin is catalyzed by firefly luciferin in the sample to oxidize luciferin and emit yellow-green light, and the expression of firefly luciferin in the sample can be detected by the chemiluminescence instrument.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Lysis Buffer	25 mL × 1 vial	50 mL × 1 vial	-20℃, 12 months
Reagent 2	Buffer Solution	7 mL × 1 vial	14 mL × 1 vial	-20℃, 12 months shading light
Reagent 3	Substrate	Power × 2 vials	Power × 4 vials	-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 working solution:
Dissolve one vial of substrate with 3 mL of buffer solution, mix well to dissolve. Aliquoted storage at -20°C for 1 month protected from light.

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: According to the table below, the corresponding volume of lysis buffer was added. Place on the ice box and mix well every 5 min, lyse for 10 min.
Suspension cells: Centrifuge at 500×g for 3 minutes at 4℃ to remove

insoluble material. According to the table below, the corresponding volume of lysis buffer was added. Place on the ice box and mix well every 5 min, lyse for 10 min. Centrifuge at 10000×g for 10 minutes at 4℃ to remove insoluble material. Collect su pernatant and keep it on ice for detection.

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

The key points of the assay

① The 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 10 samples.

Operating steps

① Sample well: add 20 μL of samples into the corresponding well.

② Add 20 μL of working solution into sample wells, and mix fully with chemiluminescence immunoassay analyzer for 2-3 s. Measure the luminescence values of each well.

Calculation

$$\text{Experiment group} = F_1 - F_3$$

$$\text{Control group} = F_2 - F_3$$

[Note]

F_1: The luminescence values of experiment group.

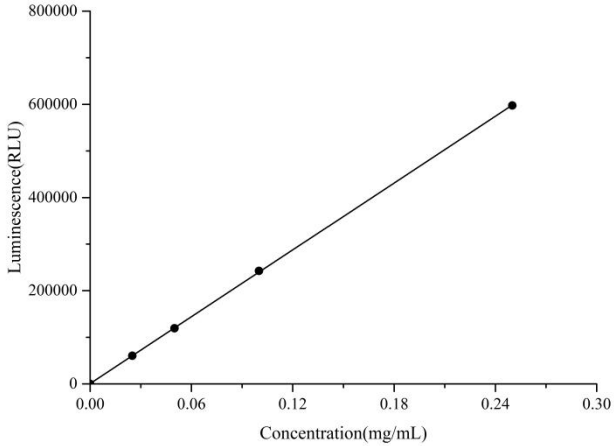
F_2: The luminescence values of control group.

F_3: The luminescence values of blank group.

Appendix I Performance Characteristics

Firefly luciferase reaction curve of:

Concentration (mg/mL)	0	0.025	0.05	0.1	0.25
Luminescence value	67	61286	114251	231454	563113
	89	59434	124583	253566	632215
Average luminescence value	78	60360	119417	242510	597664
Absoluted luminescence value	0	60282	119339	242432	597586

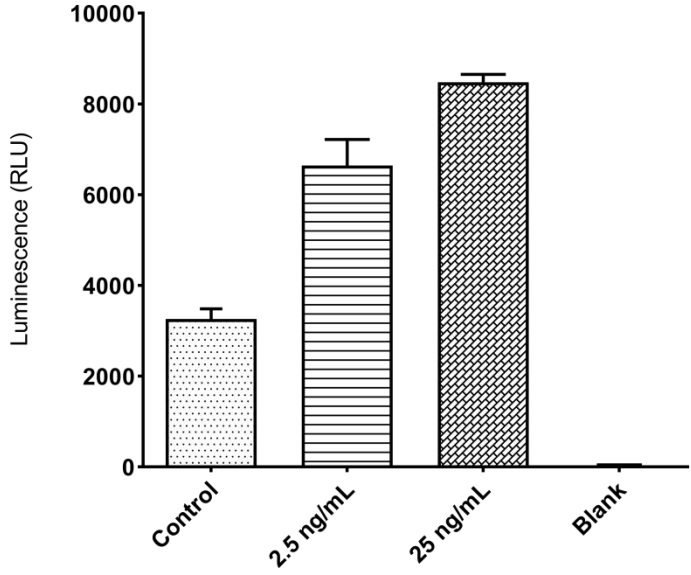


Appendix Π Example Analysis

Example analysis：

To detect the effect of tumor necrosis factor (TNF-α) induced stimulation on the expression of firefly luciferase NF-KB response element plasmids:

293T cells were inoculated in 24-well plates, about 5×10^4 cells per well, and cultured overnight. Transfection reagent containing pNF-KB-luc plasmid was added, and the cells were cultured for 24 h after observation of good state. Different concentrations of TNF-α were added to stimulate induction for 5 h. Refer to the instructions to measure the chemiluminescence value, the result is as follows.



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.