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V 1.0.0

GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit

Product Information:

Product Number	Product Name	Specification
GM-040513A	GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit	10 mL（100T）
GM-040513B		10 × 10 mL（1000T）
GM-040513C		100 mL（1000T）
GM-040513D		10 × 100 mL （10000T）

Detection Principle:

Firefly luciferase is a protein with a molecular weight of approximately 61 kDa, which catalyzes the oxidation of luciferin to oxyluciferin in the presence of ATP, magnesium ions, and oxygen. During the oxidation process, it emits bioluminescence at a wavelength of around 560 nm.

The detection principle is shown in the figure:

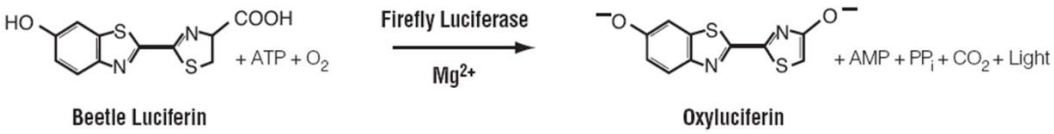

Figure 1：The detection principle diagram of firefly luciferase

Product Introduction:

The GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit (referred to as GMOne-Step 2.0) of Genomeditech is characterized by its simplicity of operation and high signal intensity. There is no need to wash or collect cells before the experiment; the reagent can be directly added to the culture medium, allowing for both cell lysis and detection to be completed in one step, saving experimental time. This kit strikes a relative balance between signal strength and stability, maintaining a relatively high signal intensity while ensuring that the signal remains relatively stable over a certain period. This results in accurate and reliable experimental outcomes, with a half-life typically exceeding 1 hour.

This product has a very wide linear range for sample measurement. In a 96-well plate, it can show a good linear relationship in the range of 200,000 cells. The detection results of the different cell amounts of the transfected firefly luciferase reporter gene plasmid in the Luc Raji cell line are shown in Figure 2 below.

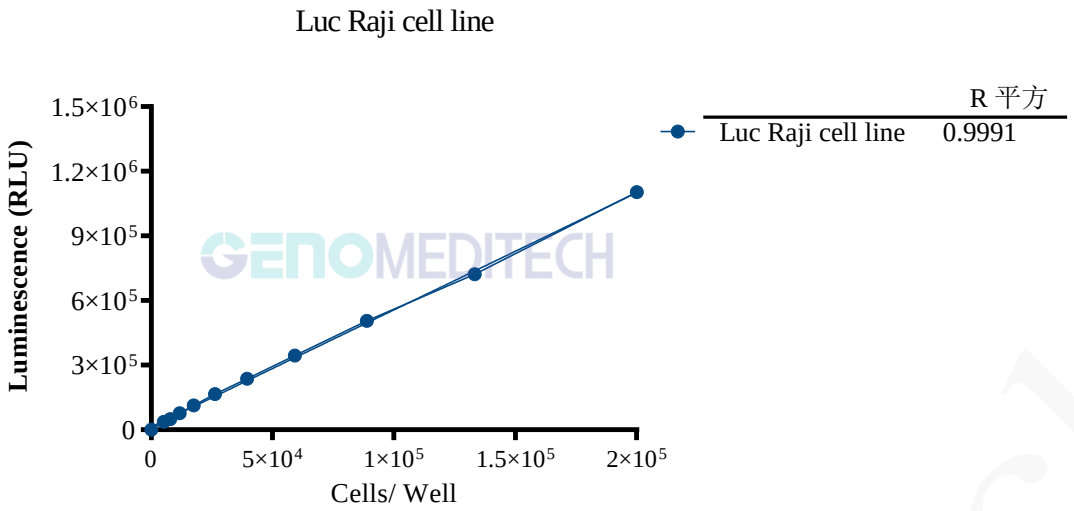

Figure 2：This product detects different cell amounts of the transfected firefly luciferase reporter gene plasmid in the Luc Raji cell line. The initial cell count in the first well is 2E5 (in suspension), followed by a series of 10 gradients diluted continuously at 1.5 times, with the final well containing 5.2E3 cells. Each gradient has 3 replicates, and the average values are taken. Actual readings may vary due to various reasons, and the data shown in the figure is for reference only.

Transportation and Reconstitution:

Transport with dry ice, and allow to fully return to room temperature before use.

Storage Conditions:

Store protected from light at -80ºC; the unopened reagent is valid for one year. If stored at -20ºC and protected from light, it is recommended to use within two weeks. After opening, it is recommended to aliquot (protected from light). Do not store at room temperature for extended periods.

Experimental Preparation:

1. Main Experimental Materials and Equipment: A 200 μL pipettor or multichannel pipette; opaque white or black microtiter plates; a multifunctional microplate reader or any instrument capable of detecting bioluminescence.
2. Reaction Temperature: Enzymatic reactions are sensitive to temperature. Please equilibrate the cell culture plates, detection reagents, and microplate reader (set to the machine's temperature) to room temperature (preferably 20-25°C) before use. The reconstitution environment for detection reagents should not exceed 25°C.

   Note: Remove the culture plates from the incubator and let them sit at room temperature for 20 minutes to equilibrate. Detection reagents must be completely returned to room temperature after thawing before use.
3. Instrument Settings: Using the Molecular Devices Spectra Max L machine as an example. PMT Setting (Detector Parameter Setting): AutoRange; Target Calibration Wavelength: 570 nm (Firefly Luciferase). Select "Shake Before Read."
4. Detection Plates: To prevent inter-well interference, it is recommended to use opaque white or black microtiter plates. If measuring high optical density values, samples can be applied in alternating wells to avoid interference.
5. Experimental Attire: Please wear laboratory coats and disposable gloves during the experiment.

Experimental Procedure (Using a 96-Well Plate as an Example):

1. Cell Lysis

1) adherent cell： It is recommended that the confluence be over 90%. There is no need to remove the culture medium; usually, add a volume of lysis reagent equal to that of the culture medium.

2) Suspension cell：As long as the cells are growing well, there are generally no density requirements. The other procedures are the same as for adherent cells.

Recommended Usage Amount

Cell Culture Dish	384-Well Plate	96-Well Plate
Volume of Culture Medium	25 µL	100 µL
Volume of Reagent to be Added	25 µL	100 µL

a) After directly adding the reagent, use a pipette to aspirate and eject 5 times to ensure thorough cell lysis. Wait for 5 minutes to allow for complete cell lysis.

b) Try to avoid the formation of foam and bubbles while pipetting.

2. Sample Loading

Aspirate 100 µL of the mixed solution (detection reagent + cell culture medium) into each well of the detection plate.

3. Fluorescence Detection

Set the parameters of the microplate reader (refer to Experiment Preparation 3). Place the detection plate into the microplate reader and shake for a few seconds. Detection can then begin.

Notes:

Temperature Effects**:** Temperature directly affects the reaction rate of luciferase. The luminous intensity and half-life depend on the reaction rate of luciferase. Therefore, before adding samples, the cell culture plates from this reagent kit should be equilibrated to room temperature to ensure consistency in the detection results. If multi-well plates are stacked, it will take longer to return to room temperature. Insufficient equilibration can lead to gradient effects between the center wells and the surrounding wells.

FAQ：

Question: Does freeze-thaw cycling affect the detection performance of the reagent kit?

Answer: Related experiments have shown that repeated freeze-thaw cycles at -70°C for up to 5 times have minimal impact on luminescence values, and stability remains largely unchanged. However, to obtain relatively better experimental data, repeated freeze-thaw cycles are not recommended.

This reagent kit is intended for research use only!