

EXOBX® Dual Luciferase Reporter Gene Assay Kit (Enhanced)

Cat. no. R4101

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Cat. No.	Reagent In Kit	Size	Storage
R4101-A	5× Cell Lysis Buffer	20 mL	-20 °C (2 years)
R4101-B	Firefly Luciferase Detection Reagent	10 mL	-80 °C (2 years)
R4101-C	Renilla Luciferase Substrate (50×)	0.2 mL	-80 °C (2 years)
R4101-D	SFAR Buffer	15 mL	-20 °C (2 years)

1. Transportation and Storage

- This product must be transported with dry ice and stored according to the specific conditions for each reagent. The shelf life is 2 years.

2. Important Information (Please read before using the kit)

- 1) Due to the temperature sensitivity of enzymatic reactions, ensure that both the test samples (cell lysates) and aliquoted Firefly Luciferase Detection Reagent are equilibrated to room temperature before performing activity measurements.
- 2) Do not freeze-thaw the Firefly Luciferase Detection Reagent repeatedly. It is recommended to aliquot it into 100 µL/tubes and store at -80°C.
- 3) Dilute 5× Cell Lysis Buffer with ddH₂O to prepare 1× Cell Lysis Buffer before use. The prepared 1× buffer can be stored at 4°C for up to 1 week or at -20°C for long-term storage.
- 4) For optimal detection, ensure that the time interval and mixing steps between adding the substrate and reading luminescence are consistent to minimize experimental error.
- 5) Renilla Luciferase Substrate (coelenterazine solution) is an ethanol-based solution and is volatile. If the volume appears lower than 0.2 mL upon receipt, add an appropriate amount of absolute ethanol to restore it to 0.2 mL.
- 6) Use the prepared Renilla Luciferase Detection Reagent immediately or store at -80°C. Do not store at -20°C as this reduces effectiveness.
- 7) If precipitation is observed in SFAR Buffer, allow it to equilibrate at room temperature until the precipitate dissolves before use.
- 8) When SFAR Buffer is added to the detection system, it deactivates Firefly luciferase activity and initiates the measurement of Renilla luciferase activity.
- 9) SFAR Buffer is exclusively used for the dual-luciferase reporter gene detection system. If Renilla luciferase activity needs to be measured separately, please purchase the EXOBX® Renilla Luciferase Reporter Gene Assay

Kit (Enhanced) (R4301). The coelenterazine dilution buffer included in that kit allows direct detection of Renilla luciferase activity with higher sensitivity.

10) For your safety, please wear a lab coat, gloves, and safety goggles while performing experiments.

11) This product is for research use only!

3. Product Introduction

This kit consists of Firefly Luciferase and Renilla Luciferase Detection Reagents, allowing for sequential measurement of both luciferases in the same sample system. The enhanced version of this Dual-Luciferase Reporter Gene Detection Kit is 3-10 times more sensitive than standard kits, making it ideal for detecting weak changes in reporter gene activity.

4. Features and Advantages

- 1) Higher sensitivity than similar commercial kits, capable of detecting luciferase activity down to 10^{-19} mol, with a linear range spanning at least 7 orders of magnitude.
- 2) Easy operation, enabling rapid dual-luciferase reporter gene detection.

5. Experimental Procedure

Adherent cells cultured in a 6-well plate are used as the experimental system. The experimental steps are as follows:

- 1) Cell transfection: Follow the instructions in the EXOBX® Plasmid DNA Transfection Reagent (Cat: R2101) manual for cell transfection. Mix the plasmid encoding Firefly luciferase and the plasmid encoding Renilla luciferase at a ratio of 10:1 (or 5:1) and transfect 2 µg per well into the cells in a 6-well plate.
- 2) Preparation of lysis buffer: Dilute 5× Cell Lysis Buffer with ddH₂O to prepare 1× Cell Lysis Buffer, which can be stored at 4°C for up to one week.
- 3) Cell lysis: At 48 hours post-transfection, discard the culture medium and wash the cells once with pre-cooled PBS. Add 0.5 mL of 1× Cell Lysis Buffer and incubate on a shaker at 4°C, gently rotating for 15-30 minutes to lyse the cells.
- 4) Sample collection: Transfer the lysate to a 1.5 mL EP tube and centrifuge at 12,000×g, 4°C for 5 minutes. Discard the pellet and transfer the supernatant to a new EP tube—this serves as the test sample (cell lysate).

5) Preparation of Renilla Luciferase Detection Reagent: Each sample requires 100 µL of Renilla Luciferase Detection Reagent. Prepare the reagent by mixing Renilla Luciferase Substrate with SFAR Buffer at a 1:50 ratio, adjusting the total volume according to the number of samples.

- 6) Activity Measurement: Turn on the detection instrument and set the measurement parameters. Typically, the delay time (integration time) is 2 seconds, and the measurement time is 10-20 seconds.
 - a. Pipette 100 µL of Firefly Luciferase Detection Reagent into an EP tube (or a 96-well plate).
 - b. Add 2-10 µL of the test sample (cell lysate) and mix quickly.
 - c. Measure the Firefly luciferase activity.
 - d. In the same detection system, add 100 µL of Renilla Luciferase Detection Reagent, mix quickly, and measure the Renilla luciferase activity.

- 7) Data Analysis: Organize and analyze the ratio of Firefly luciferase activity to Renilla luciferase activity to evaluate changes in target gene expression

Table 1. Recommended Cell Lysis Buffer Volumes

Plate Type	Lysis Buffer Volume
96-well plate	100 µL/well
24-well plate	200 µL/well
12-well plate	250 µL/well
6-well plate	500 µL/well

6. Performance Evaluation

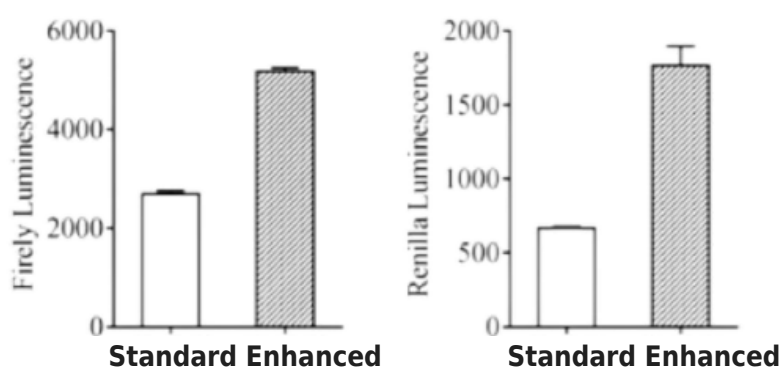


Fig. 1. Enhanced Dual-Luciferase Reporter Gene Detection Kit Performance Evaluation. The kit enables highly sensitive detection of Firefly and Renilla luciferase activities in various experimental setups.

7. Troubleshooting

Issue	Possible Cause	Solution
Low Luciferase Activity	Low cell transfection efficiency; low luciferase expression level.	Increase cell transfection efficiency or use cell lines with higher transfection efficiency, such as 293T cells.
	Insufficient amount of lysate used.	Increase the amount of lysate used in the detection system.
	Incomplete cell lysis.	Use 1× Cell Lysis Buffer for 30 minutes, ensuring continuous shaking during the lysis process.
	Luciferase detection reagent has lost activity.	Ensure proper storage and use of the kit reagents.
	Repeated freeze-thaw cycles of the luciferase detection reagent.	Aliquot Firefly Luciferase Detection Reagent into 100 µL/tubes and store at -80°C. Use freshly prepared Renilla Luciferase Detection Reagent and avoid repeated freeze-thaw cycles.
High Variability in Repeated Measurements	Sample and detection reagents were not equilibrated to room temperature.	Before detection, equilibrate test samples and Firefly Luciferase Detection Reagent at room temperature for a few minutes, avoiding light exposure.
	Inaccurate sample pipetting.	Use a high-precision pipette to ensure consistent sample volume, mixing times, and time intervals.
	Detection reagents were prepared from different batches.	Repeated freeze-thaw cycles reduce reagent sensitivity. Ensure that detection reagents used for the same sample are from the same preparation batch and have not undergone repeated freeze-thaw cycles.
High Luciferase Activity	High cell transfection efficiency; high luciferase expression level.	Reduce the amount of cell lysate used or dilute the lysate with 1× Cell Lysis Buffer before detection.
	Excessive amount of lysate used.	Reduce the volume of lysate used in the detection system.