

EXOBX® Renilla Luciferase Reporter Gene Assay Kit (Enhanced)

Cat. no. R4301

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Cat. No.	Reagent In Kit	Size	Storage
R4301-A	5× Cell Lysis Buffer	20 mL	-20 °C (2 years)
R4301-B	Renilla Luciferase Substrate (50×)	0.2 mL	-80 °C (2 years)
R4301-C	Dilution Buffer for Renilla Luciferase Substrate	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) Renilla luciferase reporter gene detection is affected by temperature; therefore, the test samples (cell lysates) and the prepared Renilla luciferase assay reagent should be equilibrated to room temperature before activity measurement.
- 2) The substrate for Renilla luciferase, coelenterazine solution, is an ethanol solution and is highly volatile. If the volume is found to be less than 0.2 ml upon receipt, an appropriate amount of anhydrous ethanol can be added to restore the volume to 0.2 ml.
- 3) Dilute the 5× Cell Lysis Buffer with ddH₂O to prepare a 1× Cell Lysis Buffer before lysing the cells.
- 4) If the prepared Renilla luciferase assay reagent is not used immediately, store it at -80°C. Do not store at -20°C, as this may lead to reduced reagent activity or inactivation.
- 5) To achieve optimal measurement results, when using a single-tube luminometer for activity detection, ensure that the interval time and mixing frequency when adding the substrate to each sample are consistent to minimize operational errors. When using a multifunctional chemiluminescence microplate reader, it is recommended to add all the samples first and then uniformly add the firefly luciferase detection reagent.
- 6) If used in combination with firefly luciferase to form a dual-luciferase detection system, please use a specific Renilla luciferase substrate dilution buffer that inactivates firefly luciferase activity (provided in the Dual-Luciferase Reporter Gene Assay Kit as SFAR buffer)
- 7) For your safety, please wear a lab coat, gloves, and safety goggles while performing experiments.**
- 8) This product is for research use only!

3. Product Introduction

This product is an enhanced Renilla luciferase reporter gene assay kit, with a sensitivity 3 to 10 times higher than that of standard Renilla luciferase assay kits. It is suitable for detecting samples with low Renilla luciferase expression or weak enzymatic activity

4. Features and Advantages

- 1) This reagent has higher sensitivity than commercially available similar reagents and can effectively detect Renilla luciferase activity.
- 2) This assay kit is easy to use and enables rapid detection of Renilla luciferase activity

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. Transfect 2 µg per well the plasmid encoding Renilla luciferase 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 R4301-C 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. Pipette 100 µl of Renilla luciferase assay reagent into an EP tube (or a 96-well plate), then add 5-20 µl of the test sample (cell lysate), mix well, and proceed with instrument detection

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

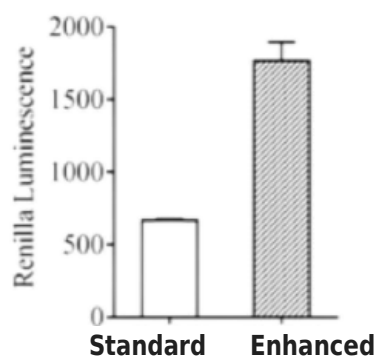


Fig. 1. Enhanced Dual-Luciferase Reporter Gene Detection Kit Performance Evaluation. The kit enables highly sensitive detection of Firefly and Renilla luciferase activities in various experimental setups.

7. Troubleshooting

Issue	Possible Cause	Solution
Low Luciferase Activity	Low cell transfection efficiency; low Renilla 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.