VKEYBIO-02-2024

For Research Use Only

Not For Diagnostic Or Therapeutic Use

KeyTec® Firefly-Renilla Luciferase Detection Kit

Instruction Manual

1. Introduction

KeyTec® Firefly-Renilla luciferase Detection kit is designed for the sensitive and robust detection of Firefly-Renilla dual luciferase reporter gene assays. The Firefly reagent detects Firefly luciferase activities, enabling accurate measurement of genetic element regulation. Additionally, the Renilla reagent quenches the Firefly luminescent signal and detects Renilla luciferase activities for normalization. This normalization can control for specific or nonspecific cellular responses and transfection efficiencies.

The detection principle is based on Luminescent technology. Within the kit, D-Luciferin reacts with Firefly luciferase, generating a sensitive luminescence signal with a half-life of approximately 2 hours. Addition of the KeyTec® Renilla Luciferase Stop & reaction buffer quenches the Firefly reaction's luminescence, and simultaneously, Coelenterazine reacts with Renilla luciferase, generating a luminescence signal that can also be read within 2 hours. The process provides a highly sensitive, robust, and homogeneous assay for the detection of Firefly-Renilla luciferase reporter gene expression in mammalian cells. (Figure 1)

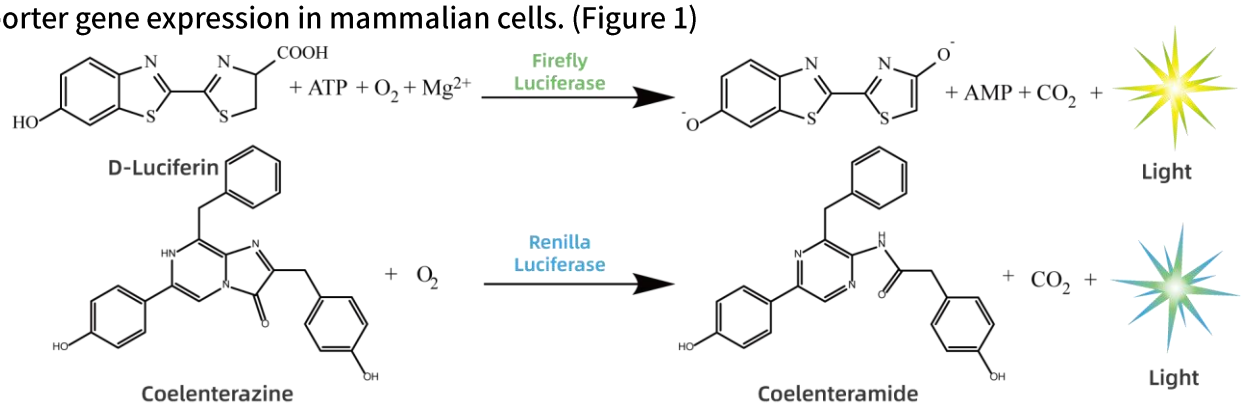

Figure 1. The Firefly-Renilla luciferase reaction of KeyTec® luciferase detection kit

Shanghai VKEY Biotechnologies Co.,Ltd.
3F, Building 2, 898 Halei Road, Pudong New District, Shanghai, China
Website：www.vkeybio.com
Email：technical-support@vkeybio.com

2. Components

CAT.	Description	Size
A2000900N	KeyTec® Firefly-Renilla Luciferase Detection Kit (100 tests)	10 mL

Firefly detection kit contains sufficient reagents to perform 100 tests of 75 µL/well.
Renilla detection kit contains sufficient reagents to perform 100 tests of 75 µL/well.

The kit contains the following components:

- 1 × 100 µL KeyTec® Firefly Luciferase Substrate 100X
- 1 × 10 mL KeyTec® Firefly Luciferase Cell lysis buffer
- 1 × 100 µL KeyTec® Renilla Luciferase Substrate 100X
- 1 × 10 mL KeyTec® Renilla Luciferase Stop & reaction buffer

CAT.	Description	Size
A2000901N	KeyTec® Firefly-Renilla Luciferase Detection Kit (1,000 tests)	2*50 mL

Firefly detection kit contains sufficient reagents to perform 1,000 tests of 75 µL/well.
Renilla detection kit contains sufficient reagents to perform 1,000 tests of 75 µL/well.

The kit contains the following components:

- 2 × 500 µL KeyTec® Firefly Luciferase Substrate 100X
- 2 × 50 mL KeyTec® Firefly Luciferase Cell lysis buffer
- 2 × 500 µL KeyTec® Renilla Luciferase Substrate 100X
- 2 × 50 mL KeyTec® Renilla Luciferase Stop & reaction buffer

CAT.	Description	Size
A2000902N	KeyTec® Firefly-Renilla Luciferase Detection Kit (5,000 tests)	2*250 mL

Firefly detection kit contains sufficient reagents to perform 5,000 tests of 75 µL/well.
Renilla detection kit contains sufficient reagents to perform 5,000 tests of 75 µL/well.

The kit contains the following components:

- 4 × 1.25 mL KeyTec® Firefly Luciferase Substrate 100X
- 2 × 250 mL KeyTec® Firefly Luciferase Cell lysis buffer
- 4 × 1.25 mL KeyTec® Renilla Luciferase Substrate 100X
- 2 × 250 mL KeyTec® Renilla Luciferase Stop & reaction buffer

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3. Storage Conditions

- Upon receipt, store the kit below -40 °C. Up to 1 years from date of receipt.
- The kit can withstand up to 10 cycles of freezing and thawing (⩾90% activity).
- When first thaw, aliquot the reagents as needed to avoid multiple freeze-thaw cycles.

4. Considerations

- Tighten the tube of KeyTec® Renilla Luciferase Substrate 100X after use due to its volatile solvent.
- The KeyTec® Renilla Luciferase Stop & reaction buffer may precipitate after freezing, but it doesn't affect performance; dissolve by shaking at room temperature.
- Prepare KeyTec® Renilla Luciferase detection reagent just before use. Discard any leftover substrate detection solution after the assay; don't store it

5. Materials Required But Not Supplied

Materials	Recommended Brand	CAT.
Cell Culture Plate (96-well, clear flat bottom, white)	Corning	3610
	Greiner	655098
White Microplates Bottom Seals	VKEY-BIO	M1000302N
Pipettes	Multiple Choices	\
Microplate Shakers	Multiple Choices	\
Microplate Reader With Luminescence	Multiple Choices	\

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6. Assay Procedure

Procedure	Stage	Operation
Step 1	Reagents Preparation	➢ **Melt the reagents:** Allow the Substrate and Cell Lysis Buffer to thaw at room temperature (not above 25 °C) before use. ➢ **Centrifuge after equilibration:** After the reagents have equilibrated to room temperature, it is recommended to centrifuge the bottles before opening the lids to concentrate the liquid at the bottom. ➢ **Prepare mixed reagent:** Mix the "KeyTec® Firefly Luciferase Substrate 100X" with the "KeyTec® Firefly Luciferase Cell Lysis Buffer" using a 1:100 volume ratio. Ensure thorough mixing to achieve the required volume of Firefly luciferase detection reagent.
Step 2	Firefly Detection	➢ **Equilibrate culture plate temperature:** Equilibrate the cell culture plate to room temperature. ➢ **Add reagent:** Add an equal volume of premixed Firefly luciferase detection reagent to the sample to be tested. (It is recommended to add 75 µL of premixed reagent to 75 µL of the cell culture to be tested.) ➢ **Shake the plate:** Shake the plate at 400 rpm for 10 minutes. Full shaking enhances the effect, ensuring thorough cell lysis and mixing. ➢ **Read Signal:** Read the luminescence signal with a microplate reader.
Step 3	Renilla Detection	➢ **Prepare mixed reagent:** Mix the "KeyTec® Renilla Luciferase Substrate 100X" with the "KeyTec® Renilla Luciferase Stop & reaction buffer" using a 1:100 volume ratio. Ensure thorough mixing to achieve the required volume of Renilla luciferase detection reagent (Prepare KeyTec® Renilla Luciferase detection reagent just before use. Discard any leftover substrate detection solution after the assay; don't store it). ➢ **Add reagent:** Add 75 µL premixed Renilla luciferase detection reagent to the sample to be tested. ➢ **Shake the plate:** Shake the plate at 400 rpm for 10 minutes. Full shaking enhances the effect, ensuring thorough cell lysis and mixing. ➢ **Read Signal:** Read the luminescence signal with a microplate reader.

Note: PBS buffer can inhibit firefly luciferase activity leading to low signal values, and PBS buffer should be avoided in the final test samples.

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7. Performance

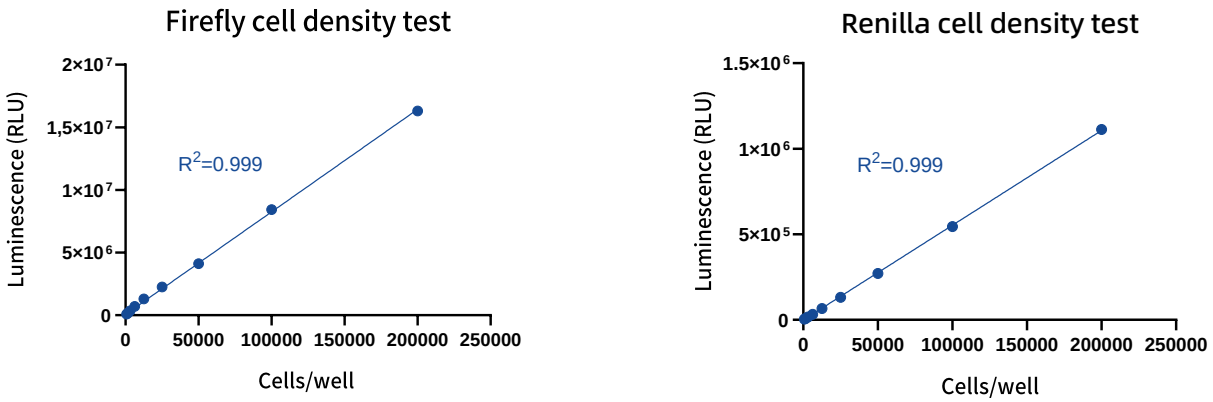

Figure 2. Correlation between Cell Number and Luminescence

Use KeyTec® Firefly-Renilla Luciferase Detection Kit to detect the overexpression of Firefly luciferase and Renilla luciferase in the HEK293 cell line. The results showed a linear relationship between the luminescence signal and the number of cells. HEK293 cells, cultured in DMEM medium with 10% FBS, were serially diluted two-fold, starting from 50,000 cells per well in a 96-well plate. Perform the assay according to the procedure outlined in Section 6. "Assay Procedure". Ten minutes after adding the Firefly luciferase detection reagent, measure the luminescence signal using the Envision's Luminescence program. (Example program details include Mirror: Luminescence, Em filter: Luminescence 700, Measurement height: 6.5 mm, and Measurement time: 1 s). Then ten minutes after adding the Renilla luciferase detection reagent, measure the luminescence signal using the Envision's Luminescence program.

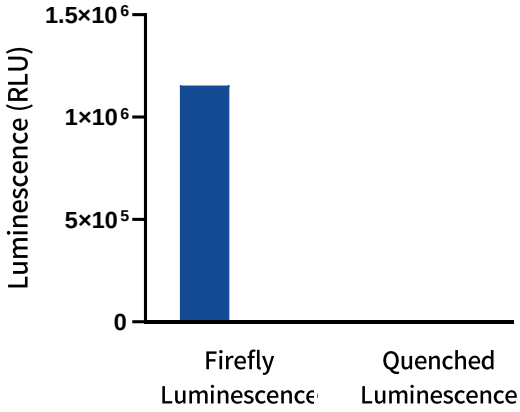

Figure 3. The quenching effect of the Renilla detection reagent on the Firefly luminescence signal.

Tip: The data provided above is for reference only. Actual results may vary depended on the performance of the microplate reader used.

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8. Data Analysis

- Depending on the assay purposes, set up positive control, test wells, negative control, blank in each assay plate. For maximal accuracy, subtract background from both Firefly and Renilla luciferase luminescence measurements.
- Firefly Background: Use non-transfected cells and KeyTec® Firefly detection reagent as the background for firefly luciferase.
- Renilla Background: Use non-transfected cells, a KeyTec® Firefly detection reagent and KeyTec® Renilla detection reagent as the background for Renilla luciferase.
- $$\text{Ratio} = \frac{\text{Firefly luminescence} - \text{Firefly background}}{\text{Renilla luminescence} - \text{Renilla background}}$$

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