VKEYBIO-01-2024

For Research Use Only

Not For Diagnostic Or Therapeutic Use

KeyTec® Ultra

Luciferase Detection Kit

Instruction Manual

1. Introduction

KeyTec® Ultra luciferase Detection kit is designed for the highly sensitive detection of Firefly luciferase activity. Simply mix the substrate with cell lysis buffer, add the mixture to the cells, and detect the Firefly luciferase signal.

The detection principle is based on Luminescent technology. Within the kit, D-Luciferin reacts with Firefly luciferase released by cells, generating a highly sensitive luminescence signal. The process provides a highly sensitive, robust, and homogeneous assay for the detection of Firefly luciferase reporter gene expression in mammalian cells. (Figure 1)

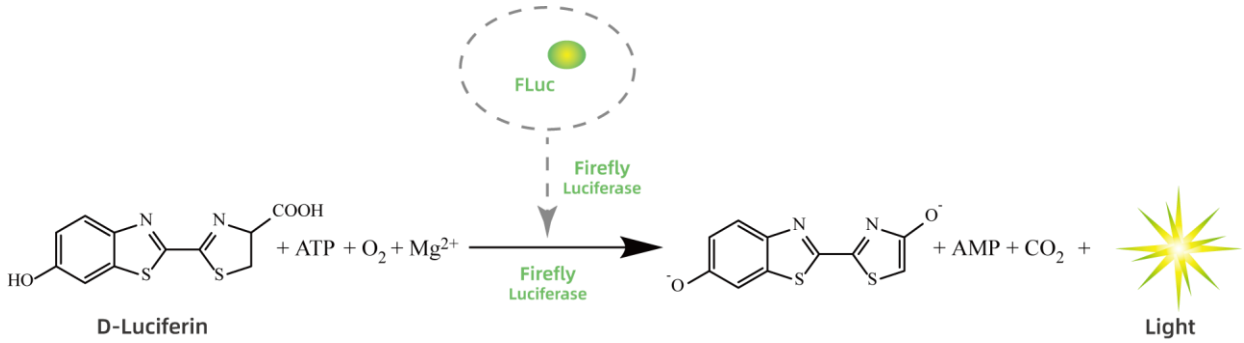

Figure 1. The Firefly luciferase reaction of KeyTec® luciferase detection kit

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2. Components

CAT.	Description	Size
A2000300N	KeyTec® Ultra Luciferase Detection Kit (100 tests)	10 mL

Each kit contains sufficient reagents to perform 100 tests of 100 µL/well.

The kit contains the following components:

- 1 × 200 µL KeyTec® Ultra Luciferase Substrate 50X
- 1 × 10 mL KeyTec® Ultra Luciferase Cell lysis buffer

CAT.	Description	Size
A2000301N	KeyTec® Ultra Luciferase Detection Kit (1,000 tests)	2*50 mL

Each kit contains sufficient reagents to perform 1,000 tests of 100 µL/well.

The kit contains the following components:

- 2 × 1 mL KeyTec® Ultra Luciferase Substrate 50X
- 2 × 50 mL KeyTec® Ultra Luciferase Cell lysis buffer

CAT.	Description	Size
A2000302N	KeyTec® Ultra Luciferase Detection Kit (5,000 tests)	2*250 mL

Each kit contains sufficient reagents to perform 5,000 tests of 100 µL/well.

The kit contains the following components:

- 5 × 2 mL KeyTec® Ultra Luciferase Substrate 50X
- 2 × 250 mL KeyTec® Ultra Luciferase Cell lysis buffer

CAT.	Description	Size
A2000303N	KeyTec® Ultra Luciferase Detection Kit (10,000 tests)	4*250 mL

Each kit contains sufficient reagents to perform 10,000 tests of 100 µL/well.

The kit contains the following components:

- 2 × A2000302N KeyTec® Ultra Luciferase Detection Kit (5,000 tests)

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3. Storage Conditions

- Upon receipt, store the kit below -40 °C. Up to 1 years.
- The kit can withstand up to 10 cycles of freezing and thawing (⩾90% activity).
- We recommend preparing the mixed reagent immediately before use.

4. 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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5. Assay Procedure

Procedure	Stage	Operation
Step 1	Reagents Preparation	➢ **Prepare 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® Ultra Luciferase Substrate 50X" with the "KeyTec® Ultra Luciferase Cell Lysis Buffer" using a 1:50 volume ratio. Ensure thorough mixing to achieve the required volume of detection reagent.
Step 2	Detection	➢ **Prepare culture plate:** Equilibrate the cell culture plate to room temperature . ➢ **Add reagent:** Add an equal volume of premixed detection reagent to the sample to be tested. (It is recommended to add 100 µL of premixed reagent to 100 µL of the cell culture to be tested.) ➢ **Shake the plate:** Shake the plate at 1200 rpm for 5 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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6. Performance

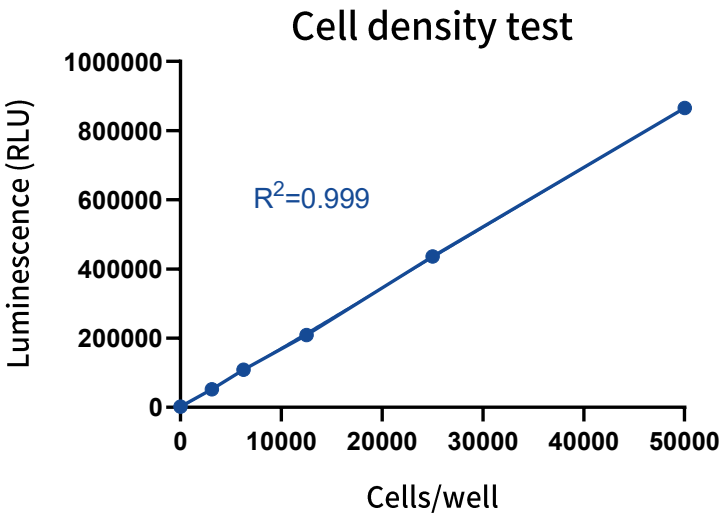

Figure 2. Correlation between Cell Number and Luminescence

Use KeyTec® Ultra Luciferase Detection Kit to detect the overexpression of Firefly 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 5. "Assay Procedure" . Five minutes after adding the 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).

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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