

VKEYBIO-02-2024

For Research Use Only

Not For Diagnostic Or Therapeutic Use

KeyTec® Luminescent 3D Cell Viability Detection Kit Instruction Manual

1. Introduction

KeyTec® Luminescent 3D Cell Viability Detection kit (3D LCV) is designed for 3D cell viability detection. It quantifies viable cells by measuring the ATP present, a key indicator of metabolically active cells. The homogeneous assay procedure involves adding a single reagent directly to 3D cells cultured in serum-supplemented medium.

The detection principle is based on Luminescent technology. Within the kit, D-Luciferin and luciferase react with ATP released by 3D cells, generating a sensitive and robust luminescent signal. With a luminescent signal half-life of 3-5 hours, this kit ensures high sensitivity and robust performance, making it an ideal choice for high-throughput screening (HTS), 3D cell proliferation and cytotoxicity assays. (Figure 1)

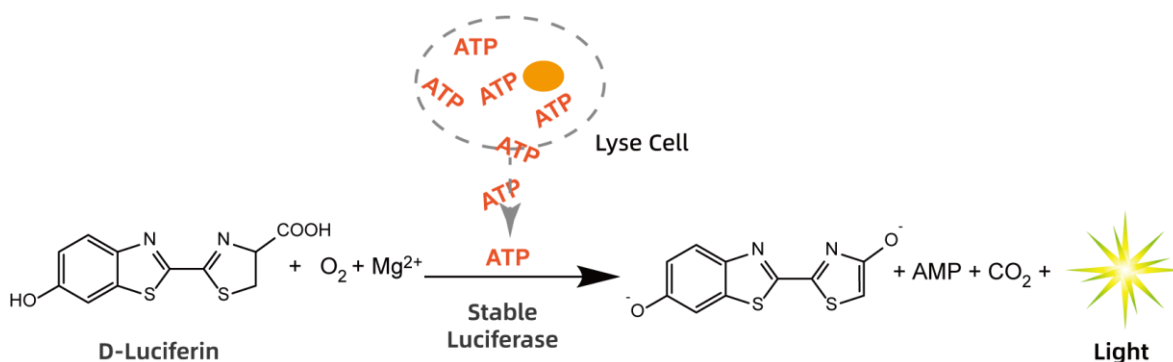


Figure 1. The luciferase reaction of KeyTec® Luminescent 3D Cell Viability detection kit

2. Components

| CAT. | Description | Size |
|-----------|---|-------|
| A2010201N | KeyTec® Luminescent 3D Cell Viability 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 × 10 mL KeyTec® Luminescent 3D Cell Viability Detection Reagent

| CAT. | Description | Size |
|-----------|---|---------|
| A2010202N | KeyTec® Luminescent 3D Cell Viability 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 × 50 mL KeyTec® Luminescent 3D Cell Viability Detection Reagent

| CAT. | Description | Size |
|-----------|---|----------|
| A2010203N | KeyTec® Luminescent 3D Cell Viability Detection Kit (5,000 tests) | 10*50 mL |

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

The kit contains the following components:

- 10 × 50 mL KeyTec® Luminescent 3D Cell Viability Detection Reagent

| CAT. | Description | Size |
|-----------|---|----------|
| A2010204N | KeyTec® Luminescent 3D Cell Viability 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:

- 2 × 250 mL KeyTec® Luminescent 3D Cell Viability Detection Reagent

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).
- ◆ We recommend preparing the mixed reagent immediately before use.

4. Materials Required But Not Supplied

| Materials | Recommended Brand | CAT. |
|---|-------------------|-----------|
| 3D Cell Culture Plate (96-well, Opaque wall) | Multiple Choices | \ |
| White Microplates Bottom Seals | VKEY-BIO | M1000302N |
| Pipettes | Multiple Choices | \ |
| Microplate Shakers | Multiple Choices | \ |
| Microplate Reader With Luminescence | Multiple Choices | \ |

5. Assay Procedure

| Procedure | Stage | Operation |
|-----------|----------------------|--|
| Step 1 | Reagents Preparation | <ul style="list-style-type: none"> ➤ Melt the reagents: Allow the reagent to thaw at 4°C or room temperature (not above 25 °C) before use. ➤ Mix reagent after equilibration: Once the reagent has reached to room temperature, invert the bottle several times to ensure thorough mixing of the reagent. (If a small amount of precipitation occurs, simply mix it several times to dissolve it, and the performance of the reagent is not affected.) |
| Step 2 | Detection | <ul style="list-style-type: none"> ➤ Equilibrate culture plate temperature: Equilibrate the cell culture plate to room temperature . ➤ Add reagent: Add an equal volume of 3D LCV reagent to the sample to be tested. (It is recommended to add 100 μL of reagent to 100 μL of the cell culture to be tested.) ➤ Shake the plate: Shake for 5 minutes to achieve thorough cell lysis and mixing, then react at room temperature for 25 minutes to reach maximum luminescent signal. ➤ Read Signal: Read the luminescent signal with a microplate reader. |

6. Performance

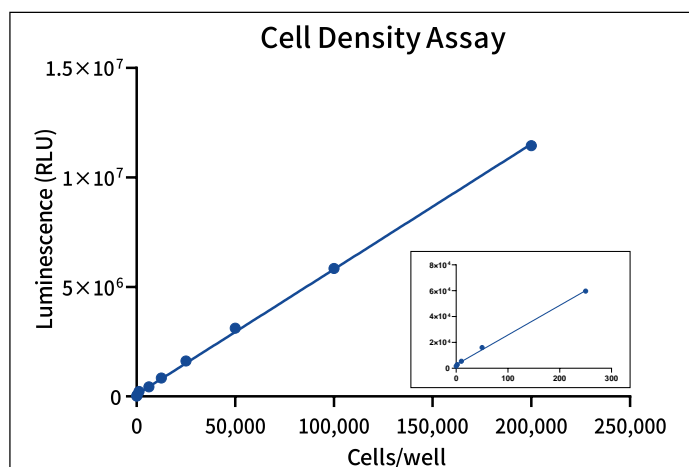


Figure 2. Correlation between Cell Number and Luminescence

Use KeyTec® Luminescent 3D Cell Viability Detection kit to detect 3D cell viability of Hela cell line. The results showed a linear relationship between the luminescent signal and the number of cells (5 - 200,000 cells/well). Hela cells, cultured in RPMI 1640 medium with 10% FBS, were serially diluted two-fold, starting from 200,000 cells per well in a 96-well plate. Perform the assay according to the procedure outlined in Section 5. “Assay Procedure” . Ten minutes after adding the reagent, measure the luminescent 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.