Cell Viability Detection Kit (Luminescence)

Catalog No.: EZB-CV1

Description

The *EZ*Bioscience® Cell Viability Detection Kit (Luminescence) provides a homogeneous method to detect cell viability or the number of viable cells in culture by quantitating the amount of ATP present, which indicates the presence of metabolically active cells. This ready-to-use reagent is based on the luciferase reaction, containing thermostable luciferase (luciferase) and high-purity luciferin (luciferin). The homogeneous assay procedure involves addition of ATP Detection Reagent directly to cells cultured in serum-supplemented medium to lyse the cells to release ATP without washing the cells and removal of medium. and the reaction shown in the figure below can occur. The reagent has excellent performance, good linear correlation, high specificity, and long signal half-life. At the same time, the ATP Detection Reagent is much more stable and will maintain > 85% light output upon storage at 4°C for several weeks.

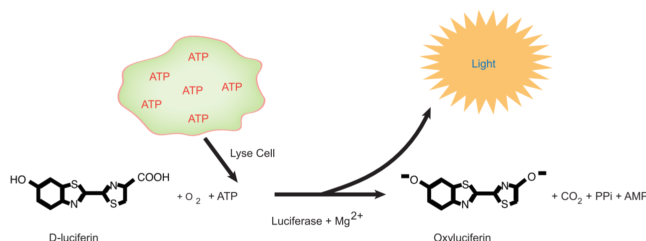

Figure 1. Overview of Cell Viability Detection Kit (Luminescence) principle.

Component

Component	EZB-CV1-S	EZB-CV1	EZB-CV1-L
ATP Detection Reagent	10 ml	100 ml	500 ml

Note: 10 ml of ATP Detection Reagent of is sufficient for 100 assays at 100 µl/assay in 96-well plates or 400 assays at 25 µl/assay in 384-well plates.

Storage

All components should be stored at -20°C and protected from light. For maximum light signal, we recommend long term storage at –80°C. The reagent can withstand four additional freeze-thaw cycles after the first thaw with no loss of light output.

User-supplied materials

single channel or multichannel pipette, 22°C water bath, opaque-walled multi-well plates adequate for cell culture, device (plate shaker) for mixing multi-well plates, microplate reader with luminescence detection module.

Protocol

A. Reagent Preparation

1. If frozen, thaw ATP Detection Reagent at 4°C overnight, or in a 22°C water bath. Do not expose the reagent to temperatures above 25°C.

Note: If the ATP Detection Reagent is stored at -80°C, do not directly transfer it into a 22°C water bath to thaw. Instead, leave the material on the bench top for 10 ~ 15 minutes and then place it in a 22°C water bath.

2. If thawing is not at room temperature, equilibrate the ATP Detection Reagent to 22°C by placing the reagent in a 22°C water bath prior to use. In a 22°C water bath, 100 ml of the thawed reagent (4°C) requires approximately 30 minutes to equilibrate, and 500 ml requires approximately 100 minutes to equilibrate to 22°C.

3. Mix gently by inverting 5 ~ 10 times to obtain a homogeneous solution before use.

B. Protocol for the Cell Viability Assay

Prepare and equilibrate the ATP Detection Reagent as described in section A prior to performing the assay.

1. Prepare opaque-walled multi-well plates with cells in culture medium. Volumes and cell number should be optimized for experimental conditions.

Note: Multi-well plates must be compatible with the luminometer used. If desired, prepare control wells containing medium without cells to determine background luminescence.

2. Equilibrate the plate and its contents to 22°C for approximately 30 minutes.

3. Add a volume of ATP Detection Reagent equal to the volume of cell culture medium present in each well (e.g., for a 96-well plate, add 100 µl of ATP Detection Reagent to 100 µl of medium containing cells).

4. Mix the contents for 2 minutes on an orbital shaker to lyse cells.

5. Incubate the plate at room temperature for 10 minutes to stabilize the luminescent signal.

6. Record luminescence. Instrument settings depend on the manufacturer. Use an integration time of 0.25 ~ 1 second per well as a guideline.

Additional Considerations

1. Temperature: The intensity and decay rate of the luminescence signal from ATP assay depends on the luciferase reaction rate. Temperature is a factor that affects the rate of enzymatic assay and therefore the fluorescence output.

Therefore, in order to maintain the consistency of the test results, it is necessary to equilibrate the reagent and cell culture to 22°C before performing the assay. It should be noted that the plates removed from a 37°C incubator and stacked tall at room temperature require longer equilibration time than single-layer plates. Insufficient balance may cause temperature gradient effects between the center and on the edge of the plates.

2. Chemical substances: The chemical compositions of different cell media will affect the rate of enzymatic assay, thereby affecting the light output. Solvents for the test compounds may interfere with the luciferase reaction and thus light output. It is recommended to set up control wells of cell culture medium containing solvents to eliminate the interference of solvents.

3. Plate: We recommend using standard opaque-walled multi-well plates suitable for luminescence detection. Opaque-walled plates with transparent bottoms can also be used, which is good for observing the cells under the microscope, but using such plates will reduce signal intensity and increase crosstalk between wells.

4. Mixing: Optimum assay performance can be obtained only when the reagent is completely mixed with the cultured cells and the cells are fully lysed. Suspension cells are easier to mix evenly than adherent cells, so omitting the mixing step after sample addition has no significant impact on the test results; if the samples to be tested are adherent cells, you can extend the vibration plate time or increase the vibration plate frequency to improve mixing and lysis efficiency. Since the pore size and liquid depth of the multi-well plates will affect the mixing efficiency, 384-well plates are more difficult to mix than the 96-well plates, so pay attention to the adjustment of the vibration plate parameters when using it. It is recommended to determine the degree of cell lysis by microscopic observation to optimize plan of the vibrating plate.

5. ATP Contamination: Strict aseptic technique is essential to prevent ATP contamination of the ATP Detection Reagent. Wear gloves and avoid contact with potentially contaminated surfaces and equipment. Use individually wrapped or designated ATP-free pipettes and pipette tips whenever possible.

Representative Results

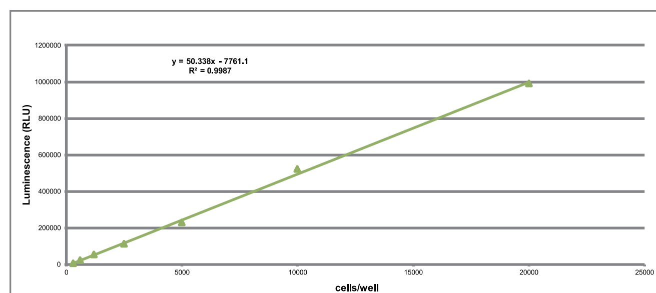

Figure 2. Cell number correlates with luminescent output. Serial twofold dilutions of A549 cells were made in DMEM with 10% FBS to prepare cell suspensions of different densities. Add 100 µl of the cell suspension of each density to a 96-well plate with opaque walls, then add 100 µl of ATP Detection Reagent to each well, mix the contents on an orbital shaker. Luminescence was recorded 10 minutes after addition of ATP Detection Reagent. There is a linear relationship (R^2 > 0.99) between luminescent signal and cell number in each plate format.

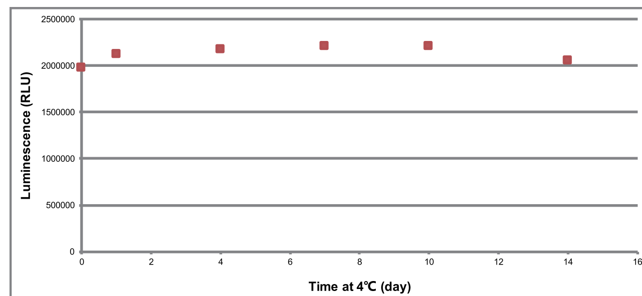

Figure 3. ATP Detection Reagent stability over time after storage at 4°C. After equilibration to room temperature, the reagent samples for different lengths of time were mixed with 10,000 cells (volumes plated were 100 µl of cells per well in a 96-well plate), mix the contents on an orbital shaker. Luminescence was recorded 10 minutes after addition of ATP Detection Reagent.

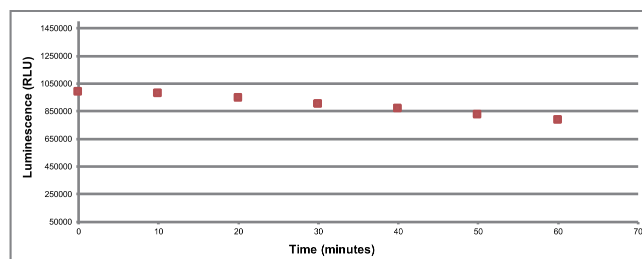

Figure 4. Luminescent signal stability detection. Add 100 µl of ATP Detection Reagent to 5,000 cells (volumes plated were 100 µl of cells per well in a 96-well plate), mix the contents on an orbital shaker. Luminescence was recorded 10, 20, 30, 40, 50 and 60 minutes after addition of ATP Detection Reagent.