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# Cell-ATP Viability Detection Kit

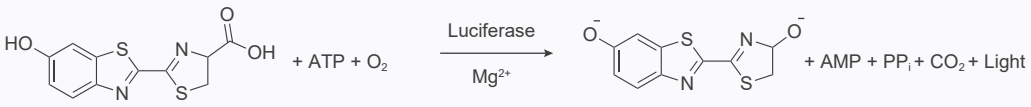
## 1 Contents

| Components | HY-K0302-10 mL | HY-K0302-100 mL | HY-K0302-500 mL |
|---|---|---|---|
| Cell-ATP Viability Detection Reagent | 10 mL | 100 mL | 500 mL |

## 2 Introduction

MCE Cell-ATP Viability Detection kit is used for detecting the number and viability of living cells in culture based on high-sensitivity bioluminescence detection technology of the ATP present.

ATP is an important index of cell metabolism which has a good linear relationship with the number of living cells. The principle of ATP bioluminescence technology is as follows: luciferase uses luciferin, adenosine triphosphate (ATP) and $O_2$ as substrates, and converts chemical energy into light energy in the presence of $Mg^{2+}$. In the luminescence reaction catalyzed by luciflucase, the concentration of ATP is linearly related to luminescence intensity within a certain concentration range. The amount of ATP is directly proportional to the number of cells present. Based on this, the Cell-ATP Viability Detection kit can be used for cell counting or viability determination by ATP content.




Luciferin Oxyluciferin

The homogeneous detection operation scheme of the Cell-ATP is "Add-Mix-Measure", which avoids the need for cell washing, medium removal and multi-step sample addition and other operations using similar detection products. Compared with MTT and CCK8, MCE Cell-ATP Viability Detection kit has the advantages of the higher sensitivity, signal stability and saving cell consumption. The reagent is compatible with commonly used media for cell culture, such as RPMI 1640, MEM, DMEM and Ham's F12, and is not affected by phenol red and organic solvents. The kit provides a more efficient and convenient solution for high-throughput screening of mobilization, cell proliferation and toxicity analysis.

## 3 Characteristics

| | |
|---|---|
| Homogeneous | "Add-Mix-Measure" format reduces the number of plate-handling steps to fewer than that required for similar ATP assays |
| Fast | Data can be recorded 10 minutes after adding the Cell-ATP Viability Detection Reagent |
| Sensitive | Measures cells at numbers below the detection limits of standard colorimetric and fluorometric assay |
| Sample saving | The number of cells required for a single detection reaction is small |
| Stability | Stable luminous signal |
| Flexible | Suitable for various multi-well plates. Data can be recorded by luminescence measurement or CCD camera or imaging device |

## 4 General Protocol

Thaw the Cell-ATP Viability Detection Reagent. The reagent and the cells should be equilibrated to room temperature prior to use. Aliquot the stock solution to routine usage volumes and store at -20°C.

Multi-well plates must be compatible with the luminometer used. The following is an example of a 96-well plate.

### 1. Cell Preparation

Perform a titration of particular cells to determine the optimal number and ensure that the working is within the linear range of the Cell-ATP Viability Detection Reagent.

Prepare opaque-walled multi-well plates with mammalian cells in culture medium, 100 µL per well for the 96-well plate, the cells number ≤ $5 \times 10^4$. Prepare control wells containing medium without cells to obtain a value for background luminescence.

Note: a) The number of inoculated cells varies with different well plates used for detection (eg. 25 µL per well for the 384-well plates (the cell number ≤ $1 \times 10^4$).

b) If drug treatment is needed, set up negative control. If the solvent content of the drug under test is high, the luciferase reaction and the light signal may be interfered. The control well of cell culture medium containing solvent can be set up to eliminate this interference.

c) Set up a cell concentration gradient during assay to determine the optimal conditions for Cell-ATP Viability Detection Reagent.

### 2. Reagents Preparation

Take appropriate amount of Cell-ATP Viability Detection Reagent and equilibrate to room temperature (eg. 100 µL per well for a 96-well plate, or 25 µL per well for a 384-well plate).

### 3. Cell Proliferation Assay

1) Equilibrated the plate for 10 minutes at room temperature.

Note: No more than 30 minutes.

2) Add 100 µL Cell-ATP Viability Detection Reagent per well for the 96-well plate (or add 25 µL Cell-ATP Viability Detection Reagent per well for the 384-well plate).

3) Mix the reagent and the cells gently for about 2 minutes.

4) Incubate for 10 minutes at room temperature to stabilize luminescent signal.

Note: Temperature gradients, uneven seeding of cells or edge effects in multiwell plates may cause the uneven luminescent signal.

5) Record luminescence. An integration time of 0.25 second per well should serve as a guideline.

6) Calculate the relative viability of the cells directly from the chemiluminescence readings, or calculate the amount of ATP from the standard curve.

Note: Different types and growth states of the cells may affect the detection data.

## 5 Storage

-20°C，one year.

Keep away from light.

## 6 Precautions

1. Aliquot the stock solution to routine usage volumes and store at -20°C.

2. Repeated freezing and thawing may cause a small amount of precipitation in the reagent. If residues remain after equilibration, they may be removed by centrifugation.

3. Please use a white or black multi-well plate (96-well plate or 384-well plate). Mutual interference may occur between adjacent wells of an ordinary transparent multi-well plate.

4. If the solvent content of the drug under test is high, the luciferase reaction and the light signal may be interfered. The control well of cell culture medium containing solvent can be set up to eliminate this interference.

5. This product is for R&D use only, not for drug, household, or other uses.

6. For your safety and health, please wear a lab coat and disposable gloves to operate.