Description

The LIVE-Step™ Cell Assay System is designed for high-throughput, homogeneous, sensitive luminescence quantification of metabolically active, viable mammalian cells. It consists of a single ready-to-use solution to lyse the cells and measure the amount of ATP present in culture in one step. Detection is linear up to >100,000 suspension cells and >50,000 adherent cells with an R^2 value greater than 0.99. The signal is stable for longer than 5 hours ($T_{1/2}$ >300 min) allowing for experimental flexibility. This product is compatible with multiple media formulations and the presence of phenol red, and stable after multiple freeze-thaw cycles.

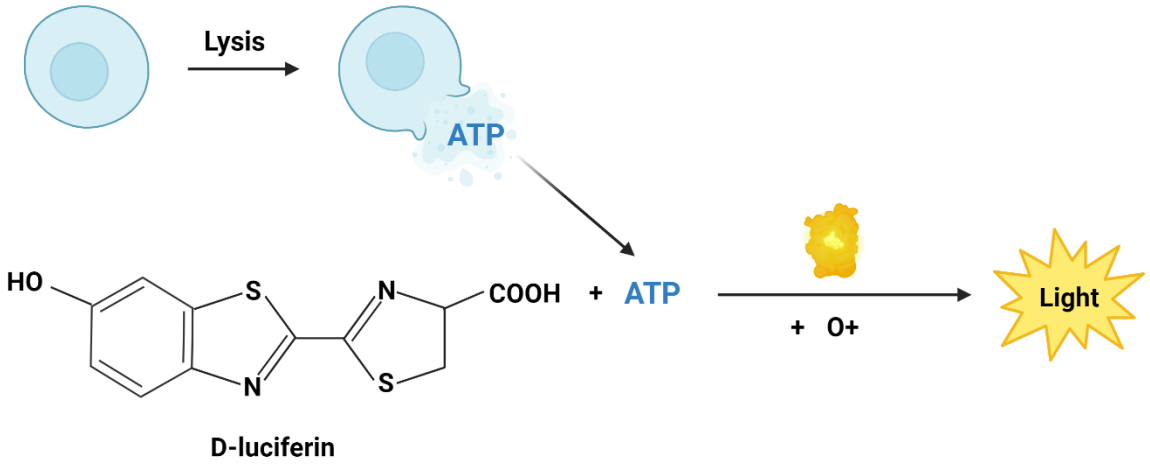

Figure 1: Luciferin activity in the presence of ATP.

Luciferin in the presence of Mg^{2+}, O_2 and ATP released from lysed metabolically active cells generates a luminescence signal. The signal generated is proportional to the number of metabolically active viable cells that were present in culture prior to lysis.

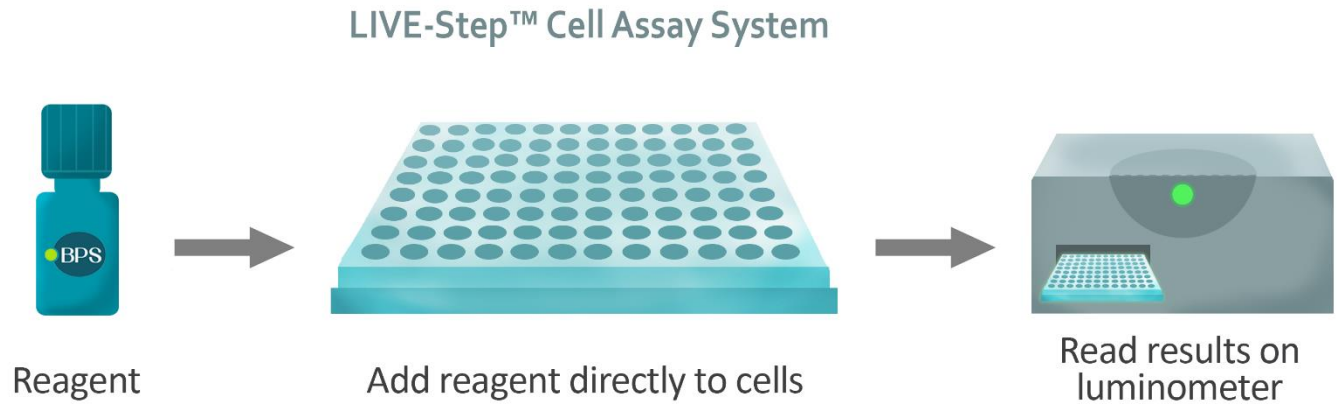

Figure 2: LIVE-Step™ Cell Assay System protocol overview.

The addition of the LIVE-Step™ Cell Solution to wells containing mammalian cells results in cell lysis and produces the luciferase-mediated reaction shown in Figure 1. The signal generated is then measured in a luminometer. The signal is proportional to the fraction of metabolically active, viable cells present in culture prior to lysis.

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Background

Determining viable cell numbers in cell culture is an essential measure across various biological fields. Traditionally, manual counting with trypan blue was the standard. While automated cell counters offer convenience, they might not be ideal for high-throughput applications. Enzyme-based methods, such as dehydrogenase assays, can provide a more scalable solution. By measuring enzymatic activity, researchers can indirectly assess cell viability. ATP-based luminescence measurements offer a direct and sensitive approach. Using thermostable firefly luciferase, this method provides a stable readout for extended periods. Compared to other techniques, ATP-based luminescence is generally more sensitive, reliable, and easier to implement, making it well-suited for high-throughput screening.

Applications

- Determine metabolically active, viable cells values in mammalian culture by using a standard curve.

Supplied Materials

Catalog #	Name	Amount	Storage
	LIVE-Step™ Cell Assay Solution	100 ml	-20°C *Protect from light*

Each kit contains sufficient reagents to quantify metabolically active viable cell number in 1000 wells of a 96-well plate, if using 100 µl/ well.

Contraindications

- The presence of organic solvents or other compounds in the culture can result in luciferase inhibition and impact the readings.
- If organic solvents or other compounds are used, it is recommended that an initial test is performed to determine the impact of the compound alone. For example, prepare a reaction containing LIVE-Step™ Cell Assay Solution, 1 µM ATP and the Diluent Reagent used to prepare the compound, and a reaction containing LIVE-Step™ Cell Assay Solution, 1 µM ATP and the compound of interest in Diluent Reagent at the maximum concentration to be tested. If a decrease in signal is observed, it may be necessary to perform a titration of the compound to assess the value at which no impact is seen, or to use a control for each concentration being tested.
- It is recommended that the edge wells of the plate are not used, to avoid temperature gradients that can affect the signal.
- ATP contamination should be avoided, by using aseptic technique while performing the assay and avoiding addition of extracellular ATP in the culture media.
- Avoid exposure of the reagent to light and excessive heat during storage.

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Materials Required but Not Supplied

- Clear-bottom, white multi-well tissue culture plates compatible with the instrument being used
- Mammalian cell culture of interest
- Cell culture medium
- Orbital Shaker
- Luminometer
- ATP Solution (Optional)

Storage Conditions

Reagents will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed. Upon first thaw, store in aliquots at -20°C. The reagent may be subjected to several freeze/thaw cycles with no effect on functionality, but it is recommended that freeze/thaw cycles be avoided whenever possible.

Safety

This product is for research purposes only and not for human or therapeutic use. Overall, this product should be considered hazardous and harmful by inhalation, in contact with skin or eyes, and if swallowed. If contact occurs, wash thoroughly.

Assay Protocol

- The following protocol is designed for a 96-well format. To perform the assay in different tissue culture formats the reagent volume should be scaled appropriately.
- Each condition should be tested in triplicate.
- LIVE-Step™ Cell Assay Solution must be at equilibrated to Room Temperature (20-25°C) before use.
- Different cell lines may exhibit variation in their lysis and/or luminescence signal and the use of LIVE-Step™ Cell Assay Solution may require optimization by the end-user.
- Adherent confluent cell lines that present contact-inhibition tend to shift their metabolism, and result in a non-linear relation between luminescence signal and viable cell number. It is recommended that over-confluency is avoided.
- To analyze multiple plates, include a common control sample in each plate and normalize the luminescence of each plate to the control contained in the same plate.
- It is recommended to include a “Cell-Free Control” to determine background luminescence. Background signal is a characteristic of luminometer performance; therefore, background luminescence must be subtracted from all readings for accuracy.

1. Thaw LIVE-Step™ Cell Solution by placing the reagent in a Room Temperature (RT) water bath.

2. Equilibrate the solution to RT and mix well before use.

3. Calculate the amount of LIVE-Step™ Cell Solution needed for the experiment.

   Note: Avoid exposing to excessive light.

4. Equilibrate the cell culture plates containing the cells to RT.

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5. Add LIVE-Step™ Cell Assay Solution directly to the culture medium, at a volume equal to the volume of the culture medium present. For example: a 96-well plate containing 100 µl of culture medium/well requires 100 µl/well of LIVE-Step™ Cell Solution.

6. Gently rock the plates for 15 minutes at RT (signal should be stable for more than 5 hours).

7. Measure luminescence using a luminometer.

8. The "Cell-Free Control" should be subtracted from all other readings.

Validation Data

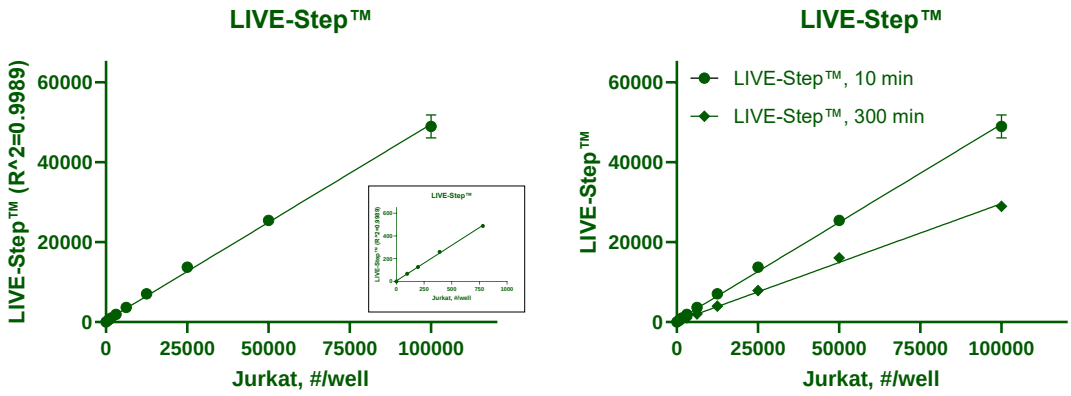

Figure 3: LIVE-Step™ Cell Assay System can be used at low cell densities without loss of sensitivity, and up to 100,000 cells/ well, with a half-time of more than 5 hours.
Left panel: Jurkat cells were plated, at different cell densities and the metabolically active viable cell fraction in culture was measured with LIVE-Step™ Cell Assay System after a 10-minute incubation period with LIVE-Step™ Cell Assay Solution. Data is shown as background-subtracted luminescence (the inlet graph shows linearity with low number of cells, <1,000 cells per well). Right panel: Jurkat cells were plated, at a broad range of cell densities and metabolic active cell fraction in culture was measured with LIVE-Step™ Cell Assay System after a 10 minutes or 300 minutes incubation period with LIVE-Step™ Cell Assay Solution. Data is shown as background-subtracted luminescence.

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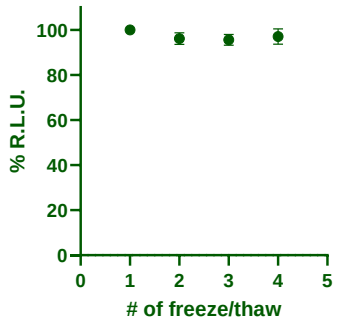

Figure 4: LIVE-Step™ Cell Assay System signal is stable up to more than 5 freeze/thaw cycles. After each freeze-thaw cycle, 100 µl of LIVE-Step™ Cell Assay Solution was added to 100 µl Thaw Medium 1 (#60187) containing 1 µM ATP in a 96-well plate, and luminescence was measured.

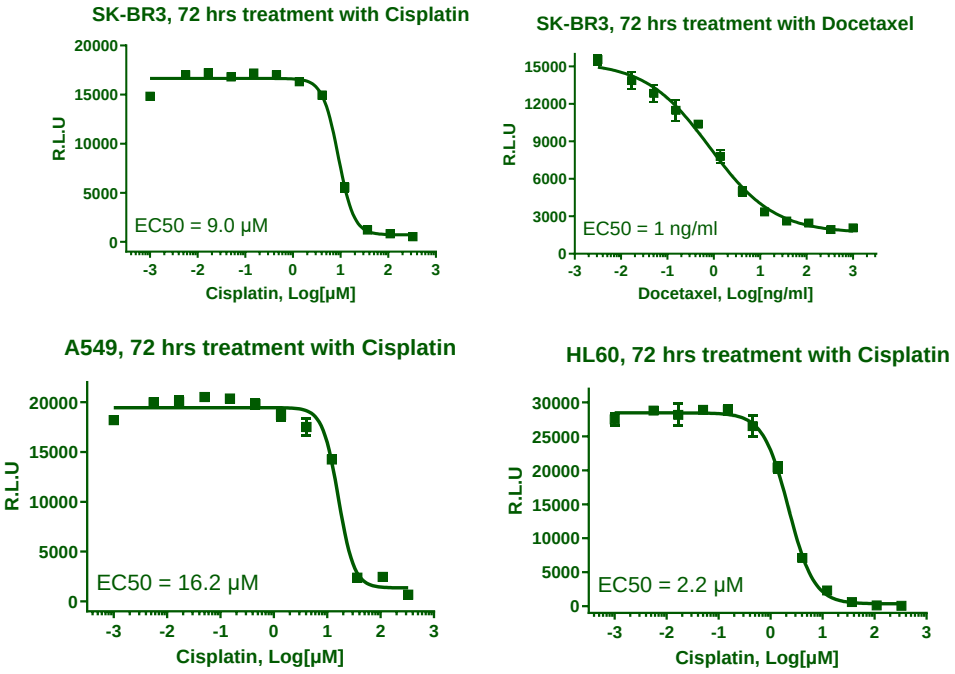

Figure 5: LIVE-Step™ Cell Assay System can be used with multiple cell lines, allowing for sensitive, reliable readouts during drug development and in vitro testing.
Top panels: SK-BR3 cells were treated with increasing concentrations of docetaxel and cisplatin for 72 hours, and metabolic active viable cell fraction in culture was measured with LIVE-Step™ Cell Assay System. Data is shown as background-subtracted luminescence. Bottom panels: HL60 and A549 cells were treated with increasing concentrations of cisplatin for 72 hours and metabolic active viable cell fraction in culture was measured with LIVE-Step™ Cell Assay System. Data is shown as background-subtracted luminescence.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for cell culture instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
ONE-Step™ Luciferase Assay System	60690	10, 100, 500 ml, 1l
TWO-Step Luciferase (Firefly & Renilla) Assay System	60683	10, 100 ml, 1l
In Vivo-Luc™ Imaging Solution	78803	5, 25 ml

Version 100324