LiveLight™ Toxicity Assay

490 BioTech

Continuous light output with automatic intensity adjustments reflecting real-time cellular health

Description

The LiveLight™ toxicity assay is a non-destructive solution for tracking cellular health in real-time. Using LiveLight™, you can assay viability continuously or repeatedly without perturbing the cells under study. Designed to combine high performance with ease-of-use, the LiveLight™ toxicity assay is provided as a single reagent mixture, pre-formulated with all the DNA and co-factors required for continuous light output. Bioluminescent output from cells treated with the LiveLight™ toxicity assay are continuously and automatically modulated by the availability of internal cellular metabolites that correlate strongly with metabolic activity levels. Signal intensity autonomously modulates to reflect metabolite availability, and therefore metabolic activity dynamics, providing a real-time report of cellular health. Healthy cells glow brightly, unhealthy cells become dim, and dead cells produce no light. The LiveLight™ toxicity assay mix can be transfected into cells using any standard method. After reagent uptake and expression, light output can be assayed repeatedly or continuously for up to one week or more, providing a live stream of cellular health data.

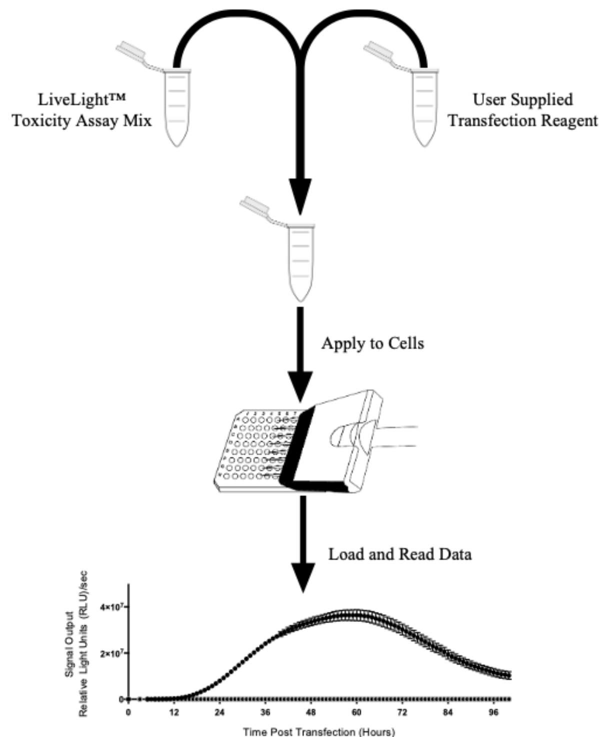

Figure 1. Overview of the LiveLight™ toxicity assay procedure.

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Disclaimers

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Principle Of Operation

The LiveLight™ toxicity assay mix contains:

1) DNA for luciferase enzyme synthesis
2) DNA for luciferin generation enzyme synthesis
3) All external co-factors required for continuous bioluminescence

The supplied mixture is transfected into the cell to introduce the DNA for transcription/translation. Simultaneously, the co-factors are delivered into the medium for cellular uptake. Immediately after translation of the luciferin synthesis portion of the system, it will begin to scavenge cytosolically available components and convert them into luciferin for the bioluminescent reaction. The luciferin synthesis system has been specifically engineered so that the scavenging process does not impact cellular health or physiology. This ensures high data integrity and prevents luciferin synthesis from interfering with native metabolic processes.

The co-expressed luciferase enzyme requires luciferin (supplied by the luciferin synthesis complex), oxygen (available in abundance under routine culture conditions), and reducing power to produce light. The availability of the reducing power component, which parallels host metabolic state, acts as the limiting reagent and allows for continuous and immediate modulation of bioluminescent intensity.

The result is a continuously adjusted bioluminescent signal from each cell in the population that reflects its real-time level of health. The more metabolically active the cell, the brighter the signal. Dead cells, which have no more metabolic activity, are incapable of producing light.

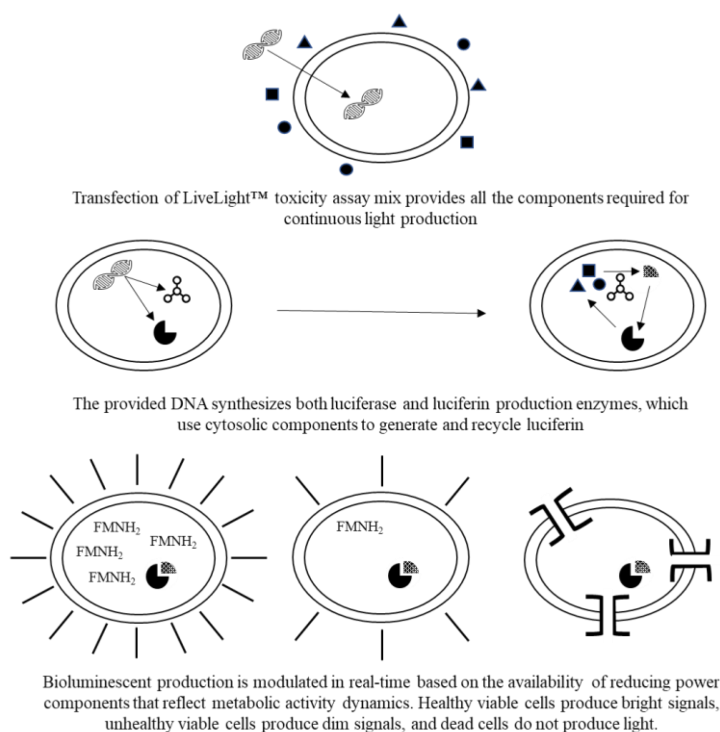

Figure 2. Overview of the LiveLight™ toxicity assay operational principle.

Kit Components And Storage Conditions

- Kit #490-01-10
  Volume: 10 µL
  Storage: -20 °C

- Kit #490-01-100
  Volume: 100 µL
  Storage: -20 °C

- Kit #490-01-1000
  Volume: 1 mL
  Storage: -20 °C

Protocol

Materials To Be Supplied By The User

- Cell culture compatible opaque-walled multi-well plates
- Transfection reagent
- Pipette and tips for reagent transfer
- Bioluminescent compatible plate reader, luminometer, or other imaging device

Cell Preparation

Because the LiveLight™ toxicity assay requires cells to express both luciferase and luciferin synthesis complex DNA for light production, assay performance is highly dependent on transfection quality. It is highly recommended that you empirically determine the optimal transfection conditions for your cell line prior to starting the assay (**Tip 1**).

Tip 1: To rapidly determine optimal transfection conditions for any cell line you can transfect LiveLight™ mix using a variety of conditions, immediately place the cells into your detection equipment, and assay every hour for 48 hours to determine which conditions produced the best output characteristics.

Cells should be prepared for transfection according to the protocol specified by your transfection reagent of choice. The LiveLight™ toxicity assay has been formulated to work with all transfection approaches.

Assay Design

Because the maximum achievable level of bioluminescence varies with transcription of the luciferase and luciferin synthesis complex genes, LiveLight™ assays should always be run with untreated controls for normalization of results. Optional controls include untransfected cells, mock transfected cells, or medium only controls (background) and vehicle controls.

Set up the desired number of replicates for your untreated controls, test samples, and optional controls. Calculate the total number of reactions using equation 1.

$$\text{Total samples} = (\#\text{ Test samples} + \#\text{ Optional controls} + 1) \times \#\text{ Replicates}$$

Equation 1. Calculating the total number of assay samples.

LiveLight™ Mix Preparation

Remove the LiveLight™ toxicity assay kit from -20 °C storage and allow to thaw at room temperature. Lightly agitate the tube to mix the solution before usage.

Once thawed, the LiveLight™ mix should be introduced into your standard transfection workflow in place of the DNA to be transfected. The LiveLight™ mix is provided with 100 ng/µL of luciferase/luciferin synthesis DNA. Generally, it is recommended that 1 µL of LiveLight™ mix be used per well for 96-well plate formats. However, it may be necessary to adjust the amount of mix used depending on the cell type to be transfected.

Use the values in Table 1 as a starting point for optimization (**Tip 2**).

Vessel Size	Scale Value
384-well	0.25×
96-well	1×
24-well	5×
12-well	10×

Table 1. Scaling the amount of LiveLight™ mix for alternative transfection formats.

After use the LiveLight™ mix should be returned to -20 °C storage (**Tip 3**).

Tip 2: The LiveLight™ toxicity assay mix can be used to make cells in culture dishes continuously bioluminescent, but the lack of opaque walls will complicate signal detection. If using culture dishes instead of multi-well plates, the use of CCD camera imaging systems is highly suggested.

Tip 3: Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. Temperature fluctuations can alter product stability.

Assaying Cells

Like any transfection, the cell needs time to uptake and express the luciferase and luciferin synthesis complex DNA before light production. After transfection, incubate cells for 24 hours under standard culture conditions (**Tip 4**).

After the 24 hour uptake and expression period, apply any necessary treatments to the cell and then transfer them to your detection equipment and begin the assay. All the external co-factors supplied by the LiveLight™ mix at the time of transfection are fully transfused into the cell within minutes after application. Therefore, it is permissible to refresh the medium at this point if directed to do so in your transfection protocol or if it is necessary to do so to apply your desired treatment. After DNA uptake is accomplished by the transfection protocol, the medium may be exchanged as often as necessary to ensure cellular health without adversely affecting bioluminescent output.

Use a 1 sec/well signal acquisition time as a starting point to optimize bioluminescent detection (**Tip 5**). The number of time points should be adjusted to match experimental need. Because light production occurs continuously and is automatically adjusted in real-time to mirror metabolic activity dynamics, you may use as many time points as required to achieve your experimental goal.

Tip 4: Some cell types begin light production in as little as 4 h. Because bioluminescence is completely self-controlled, cells can be placed into the detection equipment at any time and light will be recorded beginning at the time of production.

Tip 5: Acquisition times can be adjusted based on signal strength. Times can be as short as 100 msec for strong signals, or as long as 1 min for weak signals. Under standard conditions signal intensity is highly consistent, so longer times can be used with little change in signal variability if needed.

Data Analysis

Because transient transfection of the luciferase and luciferin synthesis complex DNA results in dynamic transcription/translation throughout the course of the assay, it is necessary to normalize assay data to the untreated control before analysis.

Use the following procedures to normalize and analyze the data obtained from the LiveLight™ toxicity assay:

Tip 6: If background subtraction is performed, use the background corrected average values in place of the raw average values when performing normalization.

Perform A Background Subtraction (Optional – only if untransfected, mock transfected, or medium only controls were used) (**Tip 6**)

1. Calculate the average value of the replicate background measurements at each time point
2. Calculate the average value for each set of replicate sample measurements at each time point
3. Subtract the average value of the background control replicate set from the average value of each sample replicate set from the same time point

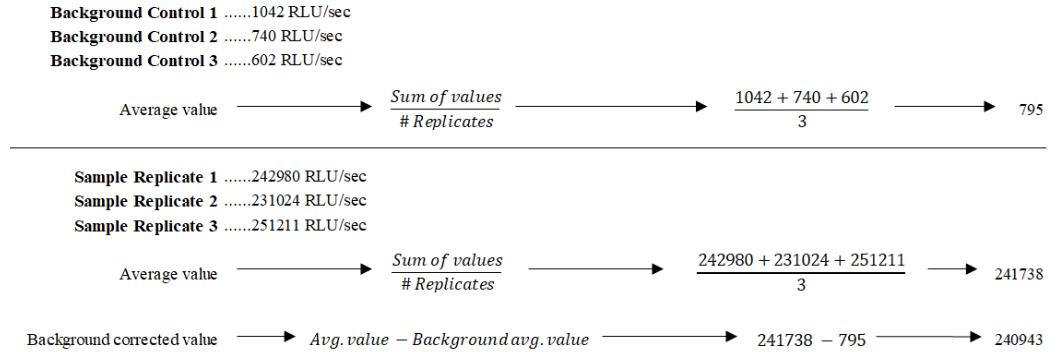

Figure 3. Example background subtraction analysis.

Normalize Data To Untreated Control

1. Calculate the average value for each set of replicate samples at each time point (**Tip 6**)
2. Divide the average value of each sample replicate set by the average value of the untreated control for that time point (**Tip 6**)
3. Multiply the obtained value by 100 to convert to percentage of the untreated control

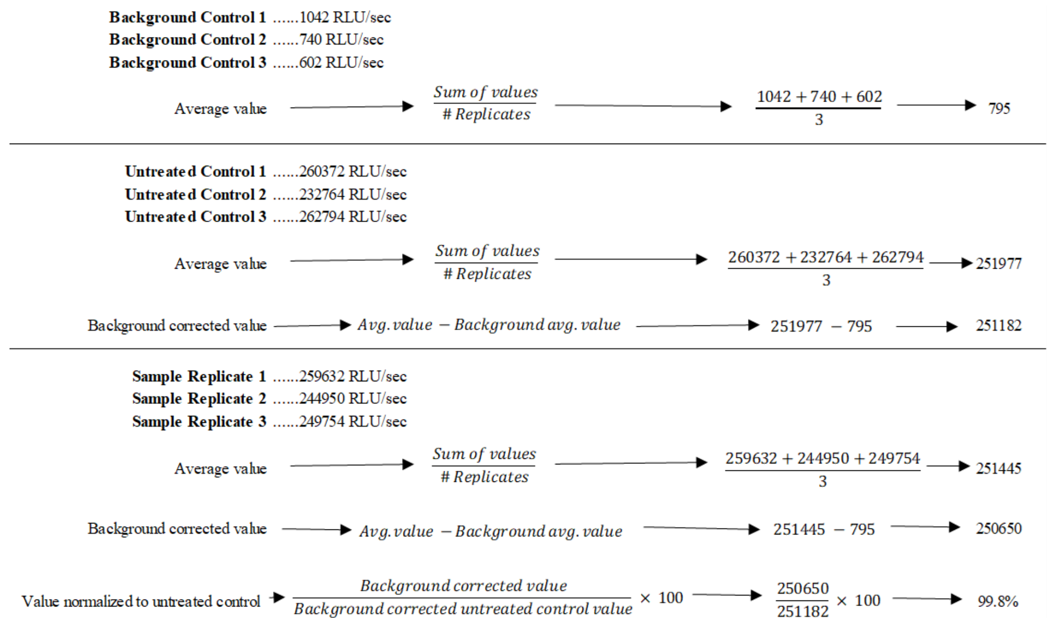

Figure 4. Example of normalizing data to untreated control values.

Normalize Data To Vehicle Control (Optional – only if vehicle controls were used)

Tip 7: To determine if the vehicle control substance was responsible for toxicity, normalize the vehicle control data to the untreated control data and analyze the results.

1. Calculate the average value for each set of replicate samples at each time point (**Tip 6**)
2. Divide the average value of your sample replicate set by the average value of the vehicle control for that time point (**Tip 6**) (**Tip 7**)
3. Multiply the obtained value by 100 to convert to percentage of the vehicle control

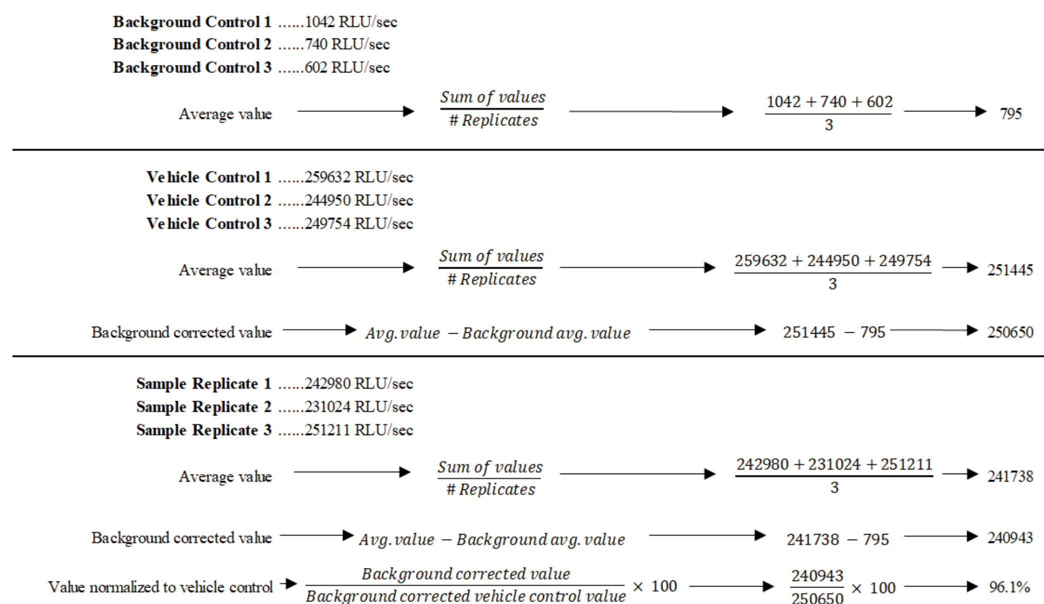

Figure 5. Example of normalizing data to vehicle control values.

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US11046962
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