LiveReport™ XRE Assay Kit

Continuous XRE monitoring with automatic intensity adjustments reflecting real-time modulation dynamics

Intended Use

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

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Description

The LiveReport™ XRE assay kit is a non-destructive solution for tracking xenobiotic response element (XRE) modulation in real-time. Using the LiveReport™ XRE kit you can assay host cell XRE modulation continuously or repeatedly without perturbing the cells under study. Designed to combine high performance with ease-of-use, the LiveReport™ XRE assay kit is provided as a single reagent mixture, pre-formulated with all the DNA and co-factors required for the cell to control bioluminescent signal output in response to XRE modulation. Cells treated with LiveReport™ XRE assay mix will express both luciferase and luciferin synthesis enzymes, so they continuously and automatically adjust their bioluminescent intensity to reflect real-time XRE modulation dynamics without any external stimulation required. The LiveReport™ XRE assay mix can be transfected into cells using any standard method. After reagent uptake and expression, XRE modulation can be assayed repeatedly or continuously for up to one week or more, providing a live stream of response data.

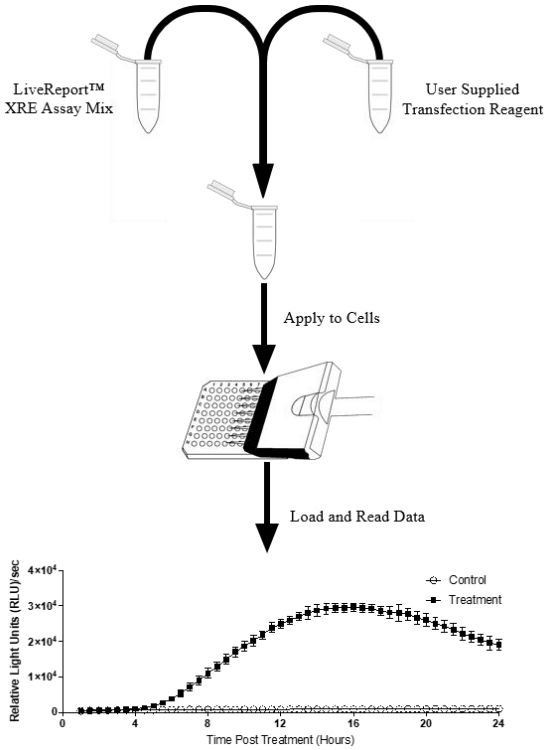

Figure 1. Overview of the LiveReport™ XRE assay procedure.

Principle Of Operation

The LiveReport™ XRE 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. Activation of the bioluminescent system is controlled by multiple XREs placed strategically upstream of a minimal promoter. When the XREs are activated, light production is stimulated. The level of stimulation correlates with bioluminescent signal intensity, making it easy to quickly determine the timing, magnitude, and duration of stimulation.

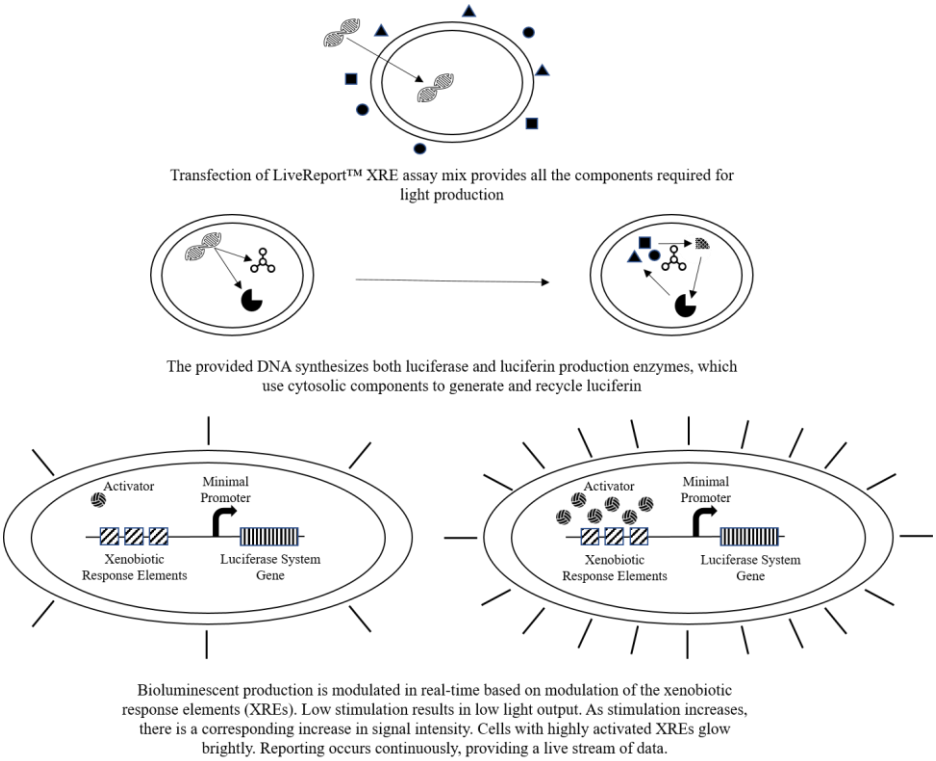

Figure 2. Overview of the LiveReport™ XRE assay operational principle.

Kit Components And Storage Conditions

- Kit #490-22-10
  Volume: 10 µL
  Storage: -20 °C

- Kit #490-22-100
  Volume: 100 µL
  Storage: -20 °C

- Kit #490-22-1000
  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

Tip 1: To rapidly determine optimal transfection conditions for any cell line you can transfect 490 BioTech's continuously bioluminescent LiveLight™ Toxicity Assay mix (Cat.# 490-01-100) using a variety of conditions, immediately place the cells into your detection equipment, and assay every hour for 48 h to determine which conditions produced the best output characteristics.

Because the LiveReport™ XRE 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**).

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

Assay Design

Because the level of bioluminescence varies with modulation of the XREs, LiveReport™ XRE assays should always be run with untreated controls for normalization of results. You may also wish to include an optional vehicle control.

Set up the desired number of replicates for your untreated controls, test samples, and, if included, optional vehicle 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.

LiveReport™ XRE Mix Preparation

Remove the LiveReport™ XRE 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 LiveReport™ XRE assay mix should be introduced into your standard transfection workflow in place of the DNA to be transfected. The LiveReport™ XRE assay mix is provided with 100 ng/µL of luciferase/luciferin synthesis DNA. Generally, it is recommended that 1 µL of LiveReport™ XRE assay mix be used per well to treat 10,000 cells/well in 96-well plate formats. However, it may be necessary to adjust the amount of mix used depending on the cell type to be transfected.

For most cell types, LiveReport™ XRE assay mix works best when provided at 0.01 ng/cell. Use this value as a starting point for optimization when working with other cell numbers.

Tip 2: Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. Temperature fluctuations can alter product stability.

After use the LiveReport™ XRE assay kit should be returned to -20 °C storage (**Tip 2**).

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 3: Need a positive induction control? We recommend 10 nM TCDD (AccuStandard #D404N)

After the 24 hour uptake and expression period, apply test compound(s) to the appropriate wells and then transfer the cells to your detection equipment and begin the assay (**Tip 3**). Note that all the external co-factors supplied by the LiveReport™ XRE assay 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.

Tip 4: 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 modulation is changing slowly.

Use a 1 sec/well signal acquisition time as a starting point to optimize bioluminescent detection (**Tip 4**). The number of time points should be adjusted to match experimental need. Because light production is continuous and automatically adjusts in real-time to reflect XRE modulation dynamics, you may use as many time points as required to achieve your experimental goal.

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 LiveReport™ XRE assay:

Normalize Data To Untreated Control

1. Calculate the average value for each set of replicate samples at each time point
2. Divide the average value of each sample replicate set by the average value of the untreated control for that time point to normalize to fold change relative to untreated control

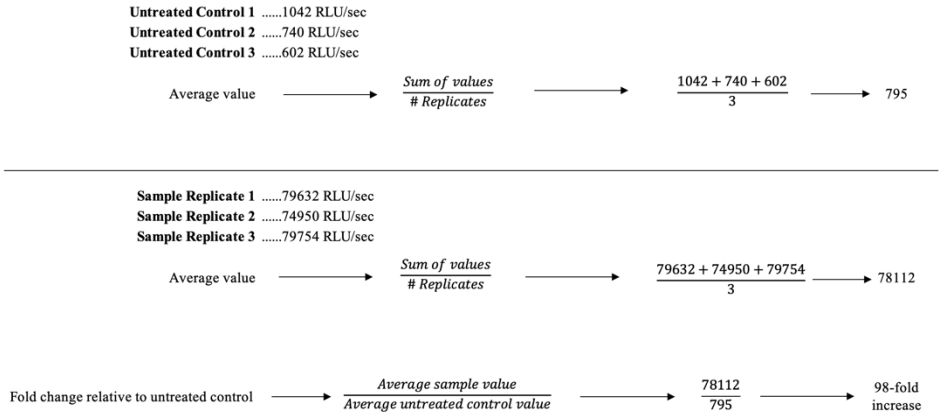

Figure 3. Example of normalizing data to untreated control values.

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

1. Calculate the average value for each set of replicate samples at each time point
2. Divide the average value of your sample replicate set by the average value of the vehicle control for that time point to normalize to fold change relative to vehicle control (**Tip 5**)

Tip 5: To determine if the vehicle control substance was responsible for modulation, normalize the vehicle control data to the untreated control data and analyze the results.

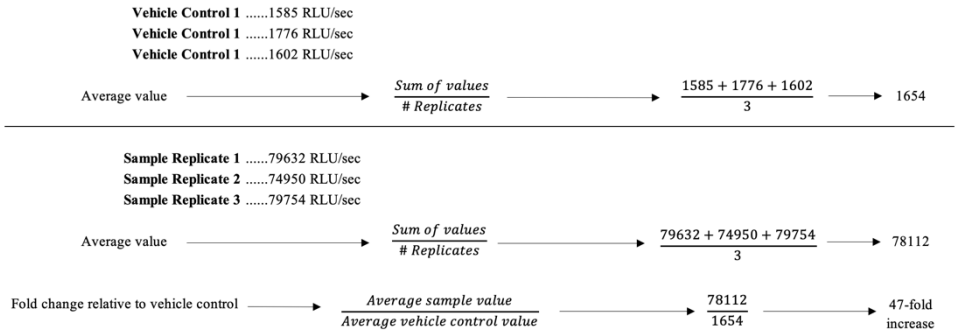

Figure 4. Example of normalizing data to vehicle control values.