Light Up Cell System (LUCS) viability assay kit

NB-63-0003

Description: Demonstration LUCS cell viability assay, sufficient reagents for 1000 determinations in 96-well plates

Updated: 03 April 2024

Kit content

LUCS solution **(1 vial)**

For research only. Not for use in diagnostic procedures.

Storage: 2-4°C, protect from light

Contents

Manufacturer's address: 74 rue des suisses 92000 Nanterre, France

info@neo-biotech.com

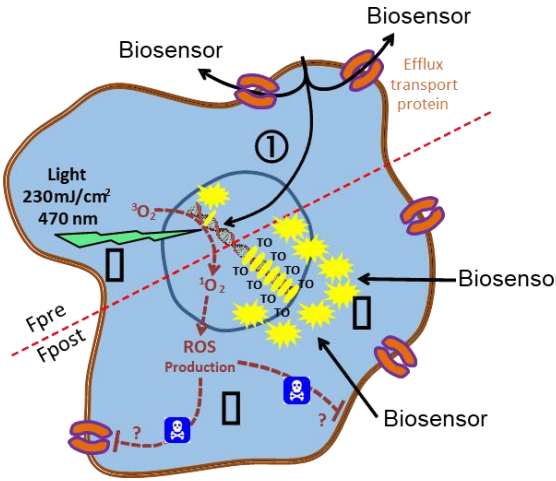
Description

The live cell Viability Assay kit is based on LUCS technology that measures the state of homeostasis or cell damage by a fluorescence readout. The technology has been optimized for high throughput on 96- and 384-well plates, suitable for commercial fluorescence readers according to a very simple protocol limited to the addition of the LUCS solution in the culture medium and two fluorescent measurements.

- For 1000 measure points in 96-well plates
- One-step procedure
- No washes
- Storage 4°C
- Time to expiration: 6 months after receipt
- Standard procedure to most immortalized cell lines, primary cells, hiPSCs, …
- Can be used on multiplexing

Mechanism

The process is called light-up cell system (LUCS) because the fluorescence level of the fluorescent biosensor increases during its photoinduction by illumination. The biosensor passively enters the cells but is quickly removed from functional cells by efflux transport proteins, resulting in a low fluorescent signal. When the light is applied, biosensor photoinduction generates intracellular ROSs, which alter the cell homeostasis or cell's ability to remove the biosensor, triggering its massive entry within the cells, and resulting in an increased fluorescence signal. If cells have been previously incubated with a toxic substance causing alteration of cellular functions, the biosensor enters massively, leading to a high fluorescence signal with consequently no increase after light application.



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Supplied Materials

Name	Amount	Storage
LUCS solution	17,5µL	4°C *for 6 months* *Protect from light*

Each kit contains sufficient reagents to perform 1000 assays in 96-well plates.

Materials Required but Not Supplied

- Cells on plate
- Appropriate cell culture medium*
- 96-well plate fluorescence reader
- AOP Illuminator if required

* We recommend the use of serum-free medium to avoid cells growing during the treatment with the toxic compound or condition

Safety 

This product is for research purposes only and not for human or therapeutic use. Potentially harmful. Avoid prolonged or repeated exposure. Avoid getting in eyes, on skin, or on clothing. Wash thoroughly after handling. If eye or skin contact occurs, wash affected areas with plenty of water for 15 minutes and seek medical advice. In case of inhaling or swallowing, move individual to fresh air and seek medical advice immediately.

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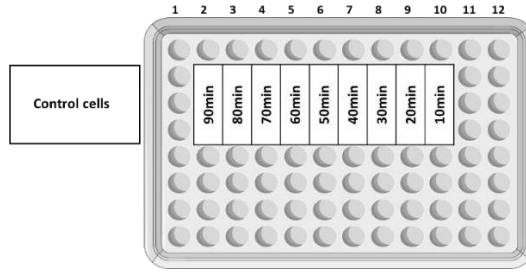
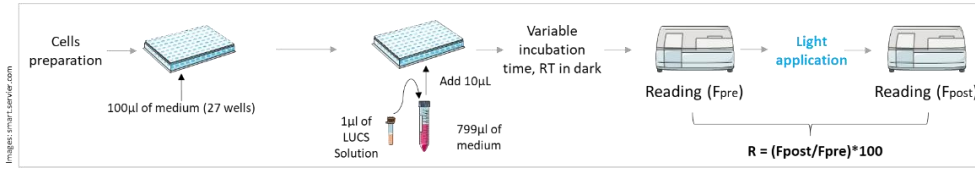
Optimization protocol (to be carried out for the use of each new cell line)

This protocol allows to find the optimized biosensor (LUCS Solution) incubation time before starting the illumination process. For this, 9 incubation times should be tested (10min, 20min, 30min, 40min, 50min, 60min, 70min, 80min and 90min).

Plate layout



Protocol



1. Remove culture medium from the wells (9 in triplicates), then add 100µL of culture medium
2. Prepare the biosensor solution: 1µL of Solution A1 + 749µL of culture medium without serum. Mix with a pipette and keep the solution protected from light
3. Add 10µL of the diluted LUCS solution in column 2 wells (condition 90min)
4. Incubate 10min at room temperature in the dark
5. Add 10µL of the diluted LUCS solution in column 3 wells (condition 80min)
6. Incubate 10min at room temperature in the dark
7. Repeat steps 5 and 6 every 10 minutes in the next columns until column 10
8. Follow step 4-6 of the **General Assay Protocol.**

Analysis

The post-illumination signal should be higher than the pre-illumination signal.
Calculate the ratio:

$$R = (F_{post}/F_{pre})*100$$

The optimized profile condition is characterized by the highest ratio. Keep the set up for routine use.

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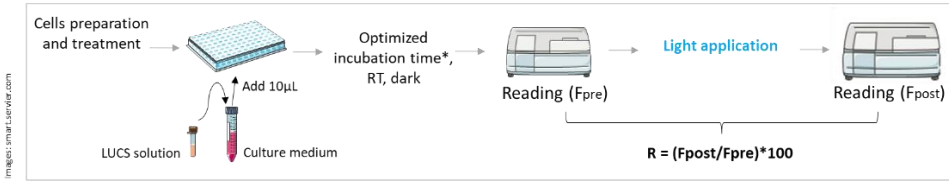
General Assay Protocol



Cell treatment with sample should be realized in medium in a final volume of 100µL/well.

1. For 96 wells, prepare 1.4µL of **LUCS solution** + 1048.6µL of culture medium. Mix with the pipette
2. Add 10µL of this diluted LUCS solution per well. ***Avoid exposing to excessive light during this step and next steps***.
3. Incubate the plate at room temperature in the dark for the optimum time determined during the optimization phase
4. Read fluorescence (F_{pre}) at the following wavelengths:

$\lambda_{Excitation}$= 505nm (±10nm)

$\lambda_{Emission}$= 535nm (±10nm)

5. Illuminate the plate using the AOP illuminator* with the "LUCS mode"
6. Wait 1 min and read fluorescence (F_{post})

N.B.: Non-specific fluorescence can be measured by reading fluorescence before step 2.

* Alternatively, follow the instructions detailed in the LUCS application note provided by the fluorescence reader's supplier.

Analysis

In each well, the ratio R of fluorescence intensities measured before (F_{pre}) and after (F_{post}) illumination is calculated as:

$$\mathbf{R= (F_{post}/F_{pre})*100}$$

Acceptance criteria: ratio value of non-toxic control should be above 100 (usually 200 to 1000); ratio values for toxic conditions should tend to 100.

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Example of LUCS Assay Results

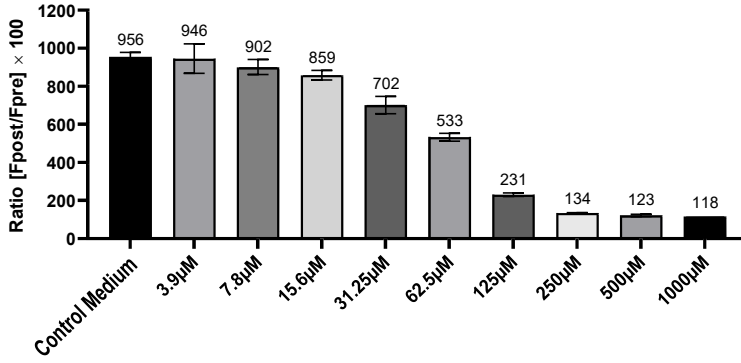

Figure 1. Dose-response values of LUCS Ratio obtained for hepatocytes (HepG2 cells) after 24h of treatment with a toxic compound (chloroquine).

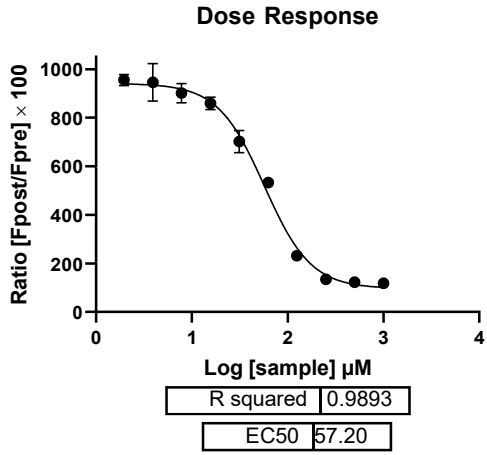

Figure 2. Dose-response curve and EC50 (µM) determination obtained after sigmoid fit by Graphpad Prism9 software.

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