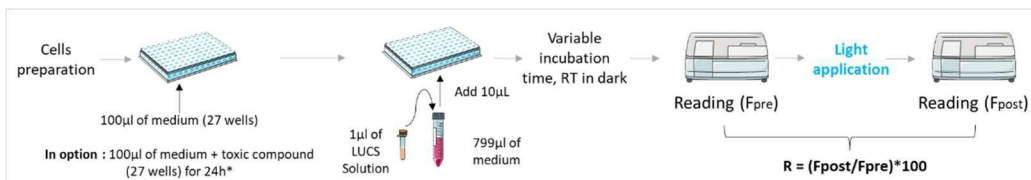


Protocol



1. Remove culture medium from the wells (9 in triplicate), then add 100µL of culture medium.
2. In option: Remove culture medium in another batch of wells (9 in triplicate) and then add culture medium supplemented with a toxic compound at a sufficient dose to kill cells. Incubate 24h* in the incubator (*this treatment time is only indicative and can be adapted according to the toxicity of the product or the toxic condition tested).
3. Prepare the biosensor solution:
 - Briefly spin the LUCS solution tube in a centrifuge to settle the drops at the bottom.
 - Add 200µl of medium in the LUCS solution tube. Mix and transfer the entire volume in a microtube.
 - Add 599µL of culture medium
 - Keep the solution protected from light
4. Add 10µL of the diluted LUCS solution in column 2 wells (condition 90min)
5. Incubate 10min at room temperature in the dark
6. Add 10µL of the diluted LUCS solution in column 3 wells (condition 80min)
7. Incubate 10min at room temperature in the dark
8. Repeat steps 7 and 8 every 10 minutes in the next columns until column 10
9. **Read fluorescence** for all wells at the following wavelengths (**Fpre**):
 - $\lambda_{\text{excitation}} = 505\text{nm} (\pm 10\text{nm})$
 - $\lambda_{\text{emission}} = 535\text{nm} (\pm 10\text{nm})$
10. **Illuminate** the plate using the AOP illuminator in **position LUCS**
11. Wait 1 min
12. **Read** fluorescence again (**Fpost**)

Analysis

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

Calculate the ratio: $R = (F_{\text{post}}/F_{\text{pre}}) * 100$

The optimized profile in nontoxic condition is characterized by the highest ratio.

In option: In wells treated with a toxic compound as positive control the signal Fpost should be close to Fpre. The ratio R should tend to 100.



Light Up Cell System (LUCS) viability assay kit

#NB-63-0003-Demo

Description: Demonstration LUCS cell viability assay, sufficient reagents for 54 determinations in 96-well plates

Update: 22March 2024

Kit content

LUCS solution (**1 vial**)

For research only. Not for use in diagnostic procedures.

Storage: 2-4°C, protect from light

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

Important Licensing Information: process cover by patents. By use of this kit, you accept the terms and conditions of all applicable Limited Use Label Licenses.

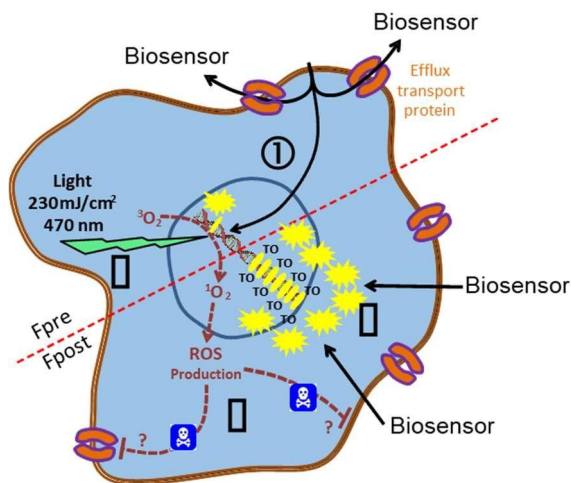
Description

The Viability Assay kit is based on LUCS technology who is a live cell test 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 54 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, ...

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 release 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 efflux or other cellular functions, the biosensor enters massively, leading to a high fluorescence signal with no increase after light application.



Supplied Materials

Name	Amount	Storage
LUCS solution	1µL	4°C for 6 months Protect from light

Each kit contains sufficient reagents to perform 54 assays in 96-well plates.

Materials Required but Not Supplied

- Cells on plate
- Appropriate cell culture medium*
- 96-well plate fluorescence reader
- AOP Illuminator might be required
- In option: positive toxic compound or condition

* 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.

Assay protocol

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). The volume of biosensor is sufficient to optionally test a toxic product in control. In this case, use a product of your choice, toxic to the chosen cell line, and apply an effective dose for a 24-hour treatment.

Plate layout

