Neostress antioxidant live cell assay

NB-63-0002

Neostress antioxidant live cell assay

#Cat: NB-63-0002 Size: 384 Well

Description: Demonstration Neostress antioxidant live cell assay, sufficient reagents for 54 determinations in 96-well plates

Update: 27 March 2024

Kit content

NeoStress solution, positive control solution (2 vials)

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

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

Neostress antioxidant live cell assay was developed from the Light Up Cell System technology that allows for fine monitoring of intracellular ROS production. The technology has been optimized for high throughput on 96- and 384-well plates, suitable for commercial fluorescence readers according to a simple protocol limited to the addition of the Solution A in the culture medium and twenty runs of illumination/fluorescence 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, …
- Can be used on multiplexing

Mechanism

The Neostress antioxidant live cell assay was developed from the Light Up Cell System technology that allows for fine monitoring of intracellular ROS production.

Neostress antioxidant live cell assay is based on the activation of an intracellular photosensitizer in a protocol that only requires a succession of light flashes and fluorescence readings. The process is called light-up cell system because the fluorescence level of the 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 ROS, 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. The increase in fluorescence is delayed or abolished in cells previously incubated with an antioxidant substance acting by neutralizing the free radicals produced by the cells under illumination.

Supplied Materials

Name	Amount	Storage
Solution A	2µL	4°C for 6 months Protect from light
Solution B	16µL	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
- Neostress Illuminator might be required

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

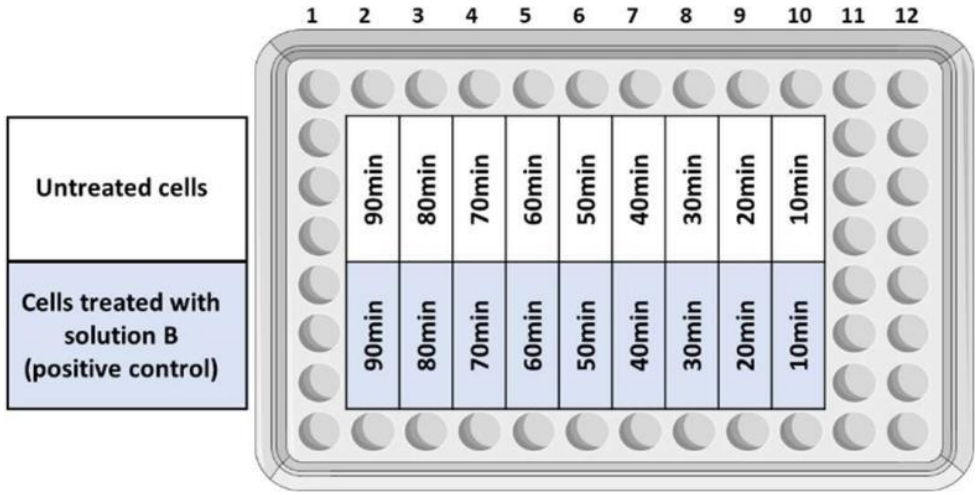
Safety ⚠

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.

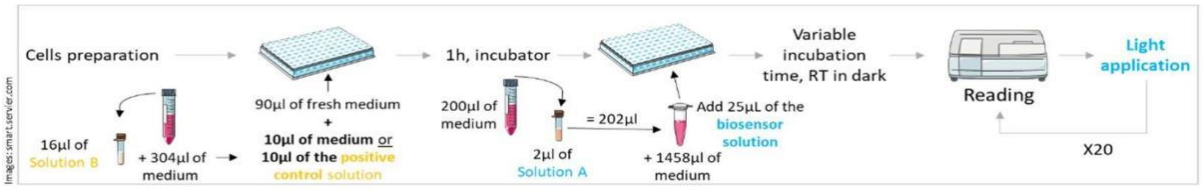
Assay Protocol

This protocol allows to find the optimized biosensor (solution A) incubation time to use for an Neostress antioxidant live cell assay in new cell line. 9 incubation times should be tested (10min, 20min, 30min, 40min, 50min, 60min, 70min, 80min and 90min) with or without the positive control treatment.

Plate layout



Protocol



1. Preparation of the positive control condition: add 304µL of culture medium in Solution B tube. Mix with the pipette.
2. Remove culture medium from all the wells, then add 90µL of fresh culture medium
3. Add 10µL of medium in “untreated” wells and 10µl of positive control solution in “positive control” wells (refer to plate layout)
4. Incubate 1h in the incubator
5. Prepare the biosensor solution:

a. Briefly spin the Solution A tube in a centrifuge to settle the drops at the bottom.
b. Add 200µl of medium in the solution A tube. Mix and transfer the entire volume (202µl) in a 2 ml microtube.
c. Add 1458 µl of culture medium
d. Keep the solution protected from light

6. Add 25µl of the biosensor solution (diluted solution A) in column 2 wells (Condition 90min)
7. Incubate 10 min at room temperature in the dark
8. Add 25µl of the biosensor solution (diluted solution A) in columns 3 wells (condition 80min)
9. Incubate 10min at room temperature in the dark
10. Repeat steps 8 and 9 every 10 minutes in the next columns until column 10
11. **Read fluorescence** for all 54 conditions at the following wavelengths:

    $\lambda_{Excitation}$= 505nm (±10nm)

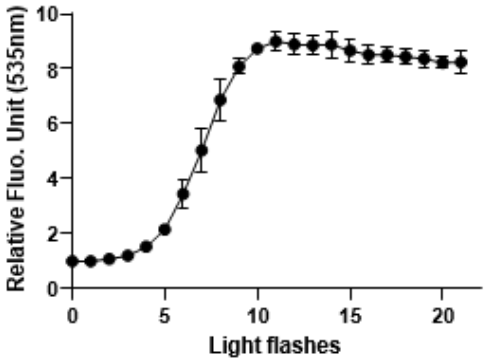
    $\lambda_{Emission}$= 535nm (±10nm)
12. **Illuminate** the plate using the AOP illuminator in position AOP1
13. Wait 1 min
14. **Read** fluorescence again
15. **Repeat steps 12 to 14 twenty times**

Analysis

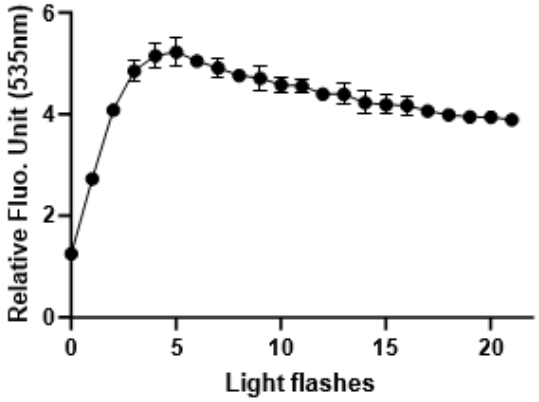
Draw the kinetics profiles for each condition.

The optimized profile is characterized by a good signal amplitude and a progressive signal increase:

Expected profile:



Wrong profile:



Avoid profiles with too rapid a rise in the signal (ideally, the signal should rise around 3-4 light flashes). It's also necessary for the final plateau to be reached before or around the 15th light flash.

In wells treated with the antioxidant as positive control (solution B), the signal should remain at pre-illumination level and not rise (assuming the antioxidant used works on the chosen cell line).