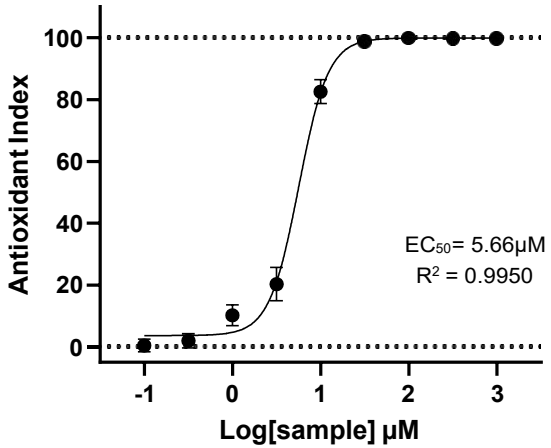

Figure 4. Dose response curve and EC_{50} determination obtained with Graphpad Prism 10 software.

AOP1 LIVE CELL ANTIOXIDANT ASSAY KIT

AOP1 Live-Cell Antioxidant Assay kit

#NB-63-0002

Description: AOP1 Live-Cell Antioxidant Assay, sufficient reagents for 400 determinations in 96-well plates

Updated: 09 April 2024

Kit content

AOP1 solution, positive control solution (2 vials)

For research only. Not for use in diagnostic procedures.

Storage: 2-4°C, protect from light

Content

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

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

info@neo-biotech.com

Description

AOP1 antioxidant live cell assay was developed from the LUCS (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 400 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 primary cells, hiPSCs, immortalized cell lines,…
- Can be used on multiplexing

Mechanism

The AOP1 antioxidant live cell assay was developed from the LUCS (Light Up Cell System) technology that allows for fine monitoring of intracellular ROS production. AOP1 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 (LUCS) 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 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 fluorescent 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	16µL	4°C *for 6 months* *Protect from light*
Solution B	20µL	4°C *for 6 months* *Protect from light*

Each kit contains sufficient reagents to perform 400 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

AOP1 LIVE CELL ANTIOXIDANT ASSAY KIT

Step 3: Antioxidant index calculation

AUCs are calculated using the formula: **AUC = $\int_0^x$NFU** with x = number of flashes needed to reach the plateau in the control condition (flash number 17 in our example).

Antioxidant Index for a sample condition "y" is calculated for each replicate using the formula:

$$\text{Antioxidant index} = 100 - 100 * (AUC_y / \text{mean}(AUC_{control}))$$

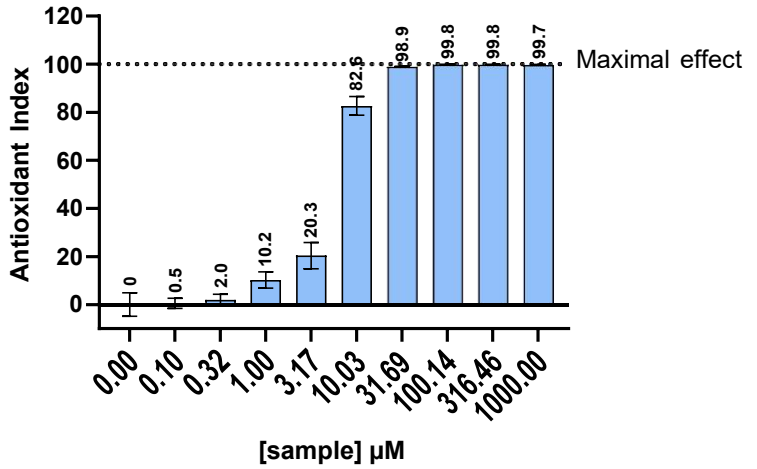

Figure 3. Antioxidant Index for each tested sample concentration. Error bars represent SD values from triplicates.

Step 4 (optional): EC_{50} Determination

Antioxidant Index (AI) values can be used to calculate efficacy concentration (EC) values from a mathematical non-linear regression model (sigmoid fit) given by Prism 10, following the equation:

$$\mathbf{Y = Bottom + (Top\text{-}Bottom) / (1 + 10^{((LogEC_{50}\text{-}X) * HS)})}$$

With HS = slope coefficient of the tangent at the inflection point

EC_{50} and R^2 values can be deduced from the regression model.

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Step 1: Raw Data plot

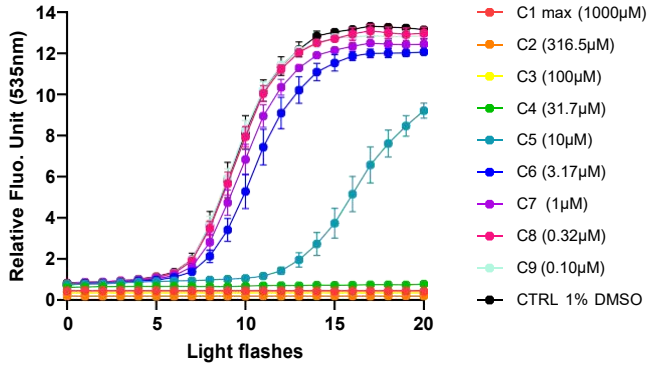


Figure 1. Kinetic raw data of an antioxidant compound in dose response mode measured in HepG2 cells.

Step 2: Normalized data

Raw data can be normalized by subtracting the fluorescence level measured before illumination:

$NFU = RFU\ FN_x - RFU\ FN_0$

with NFU = Normalized Fluorescence Unit, RFU = Relative Fluorescent Unit, FN_x = Flash Number x

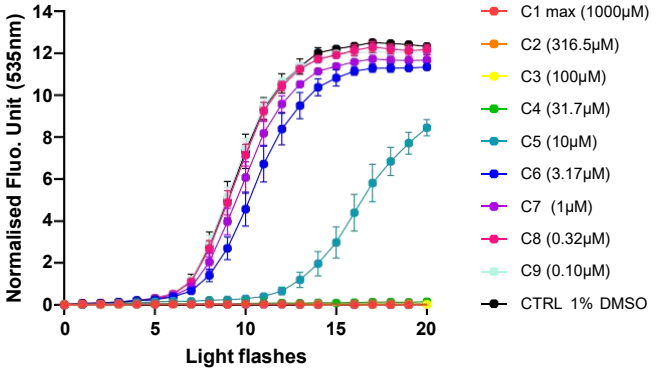


Figure 2. Kinetic profiles after data normalization.

AOP1 LIVE CELL ANTIOXIDANT ASSAY KIT

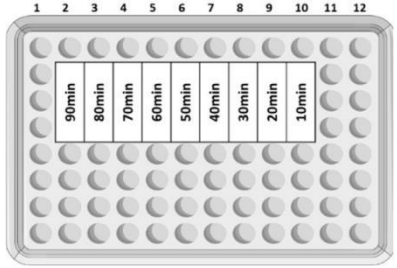
Safety

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.

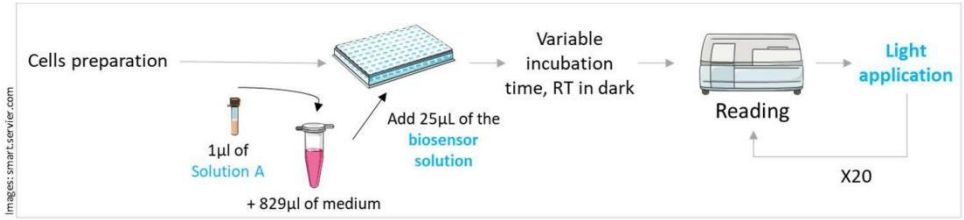
Optimization protocol (to be carried out for the use of each new cell line)

This protocol allows to find the optimized biosensor (Solution A) 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 triplicate), then add 100µL of culture medium
2. Prepare the **biosensor solution**: 1µL of Solution A + 829µL of culture medium. Mix with a pipette and keep the solution protected from light
3. Add 25µL of the biosensor solution in column 2 wells (condition 90min)
4. Incubate 10min at room temperature in the dark
5. Add 25µL of the biosensor 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 9-12 of the **General Assay Protocol**

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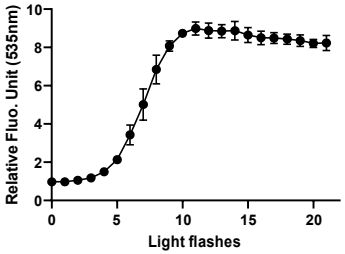
Analysis

Draw the kinetic profiles for each condition.
The optimized profile is characterized by a good signal amplitude and a progressive signal increase:

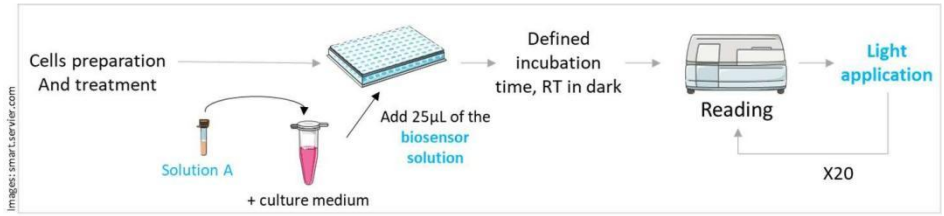
Expected profile: **Wrong profile:**



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

AOP1 LIVE CELL ANTIOXIDANT ASSAY KIT

General Assay Protocol



This protocol is given for one 96 well plate, adjust the volumes according to the desired number of plates.

1. Prepare samples 10X concentrated
2. Prepare the positive control: 4 µL of **Solution B** + 36 µL of culture medium. Mix with the pipette
3. Remove culture medium from cells, then add 90µL of fresh culture medium
4. Add 10µL of positive control and sample conditions to wells
5. Incubate for 1h in incubator
6. Prepare the **biosensor solution**: 3.13µL of **Solution A** + 2596.9µL of culture medium. Mix with the pipette and keep the solution protected from light
7. Add 25µL of this **biosensor solution** per well. ***Avoid exposing to excessive light during this step and next steps***.
8. Incubate the plate at room temperature in the dark for the time defined in the optimization phase
9. Read fluorescence at the following wavelengths:

$\lambda_{Excitation}$= 505nm (±10nm)
$\lambda_{Emission}$= 535nm (±10nm)

10. Illuminate the plate using the AOP illuminator* on “AOP1” mode
11. Read fluorescence
12. **Repeat steps 10 and 11 twenty times**

N.B.: Non-specific fluorescence can be measured by reading fluorescence before step 6.

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

Example of Data Analysis

Example of AOP1 results obtained with hepatocyte cells (HepG2) treated with an antioxidant sample. In this example, a range of sample concentrations were tested in triplicates. A control with solvent alone (1% DMSO) was added.

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