

# AOP1 SKIN

Measure of intracellular antioxidant activity in human skin cells



## ASSAY TYPE

Cell-based efficacy assay

## SPECIFICITY

Evaluation of intracellular antioxidant activity by direct measurement of neutralization of intracellular free radicals.

## STUDY SPECIFICATIONS

- Full dose-effect study (nine doses, 4-log range)
- Evaluation of efficacy concentrations (EC10, EC50, EC90)
- At least two independent experiments
- Three end-point measurements (triplicate) for each dose
- Analytical report and monographs
- Comparison with standard antioxidant resveratrol

## ASSAY PRINCIPLE

The assay relies on the controlled generation of intracellular radical species by a photo-induction process. A cell permeant biosensor is added to the cell culture medium and binds to nucleic acids with a low fluorescence level. When the biosensor is photoactivated by appropriate LED illumination, it's relaxation is accompanied by an energy transfer to the intracellular dioxygen molecule ( $^3O_2$ ) resulting in the production of singlet oxygen ( $^1O_2$ ) which in turn triggers a cascade of Reactive Oxygen Species (ROS) production including the free radical species superoxide anion ( $O_2^-$ ) and hydroxyl radical ( $OH\cdot$ ). Presence of ROS species leads to cell alteration and fluorescence level increases. Neutralization of intracellular free radicals by sample added in the culture medium inhibits this process, maintaining fluorescence emission at low level. Kinetic records allow for antioxidant index calculation. Dose-response curves fitting with sigmoid model allow for efficacy standard concentrations (EC10, EC50, EC90) evaluation (**patented technology**).

## DETECTION METHOD

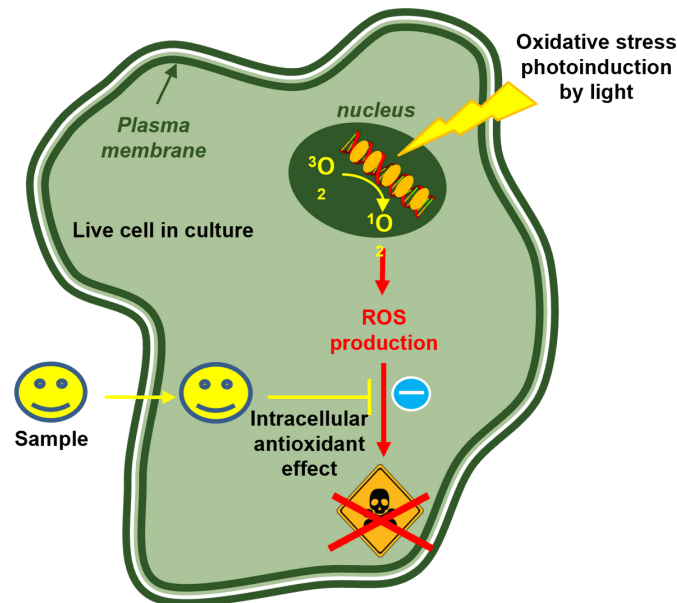
Fluorescence (exc/em 505-535 nm)

## ASSAY FORMAT

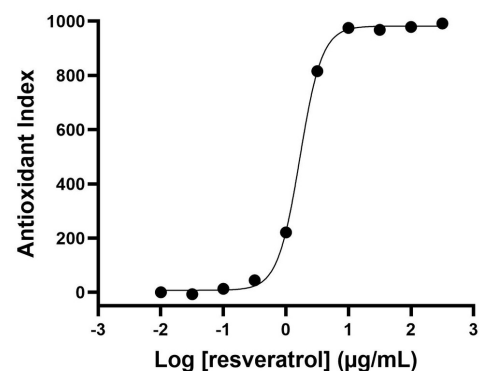
96-well cell culture plates

## CELL MODEL

Human immortalized keratinocytes (HaCaT), primary keratinocytes (NHEK), primary dermal fibroblasts (NHDF) or other cell models



AOP1 on HaCaT with 1h of resveratrol treatment



$R^2 = 0.9995$   
EC50 = 1.67 µg/ml (7.32 µM)