AOP1 SKIN

Measure of intracellular antioxidant activity in human skin cells

ANTI OXIDANT POWER

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 (3O2) resulting in the production of singlet oxygen (1O2) which in turn triggers a cascade of Reactive Oxygen Species (ROS) production including the free radical species superoxide anion (O2.-) and hydroxyl radical (OH.). 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

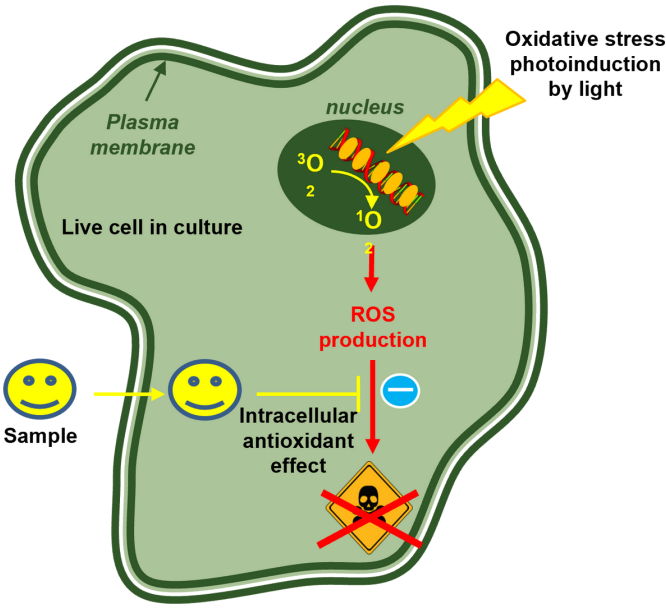
Fluorescence (exc/em 505-535 nm)

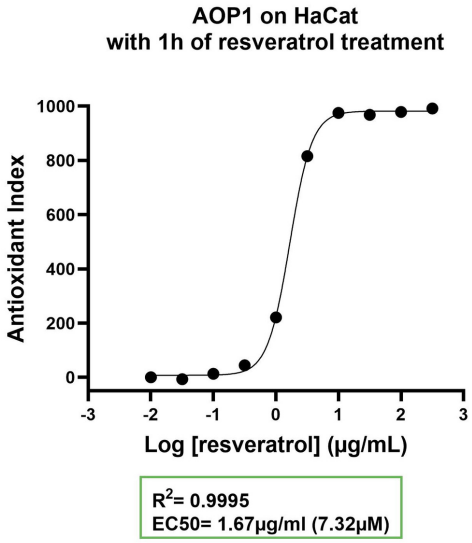
ASSAY FORMAT

96-well cell culture plates

CELL MODEL

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





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AOP2 SKIN

Induction of natural antioxidant cell defence (Nrf2-ARE pathway) in human keratinocytes

ANTI OXIDANT POWER

ASSAY TYPE

Cell-based efficacy assay

SPECIFICITY

Evaluation of natural cell defence by Antioxidant Responsive Element (ARE) induction through Nrf2 transcription factor

STUDY SPECIFICATIONS

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

ASSAY PRINCIPLE

The assay relies on the ability of tested sample to activate Nrf2 transcription factor. Under basal conditions, Nrf2 is retained to the cytosol by binding to Keap-1 protein. Activators of Nrf2 pathway lead to Keap-1/Nrf2 dissociation and translocation of Nrf2 to nucleus where it binds the Antioxidant Response Element. ARE is an enhancer sequence found in the promoter region of many genes coding for antioxidant, detoxification and cytoprotective proteins. The luminescent reporter system we use to monitor Nrf2 response contains a firefly luciferase gene under the control of ARE stably integrated into cells. Cells are incubated for 15 hours with the sample, luminescence is read after D-luciferin addition. Results are expressed as gene expression fold increase. Dose-response curves fitting with sigmoid model allow for efficacy standard concentrations (EC10, EC50, EC90) evaluation. Sulforaphane is used as a positive control.

DETECTION METHOD

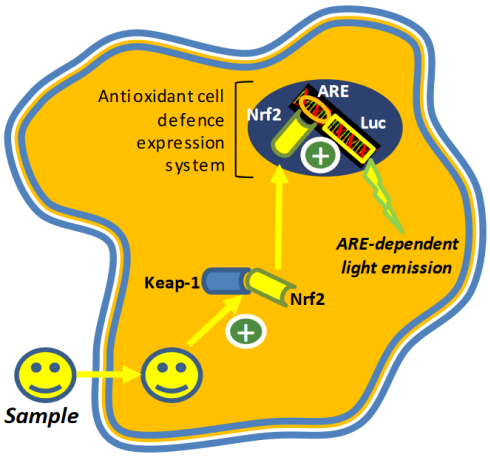
Luminescence

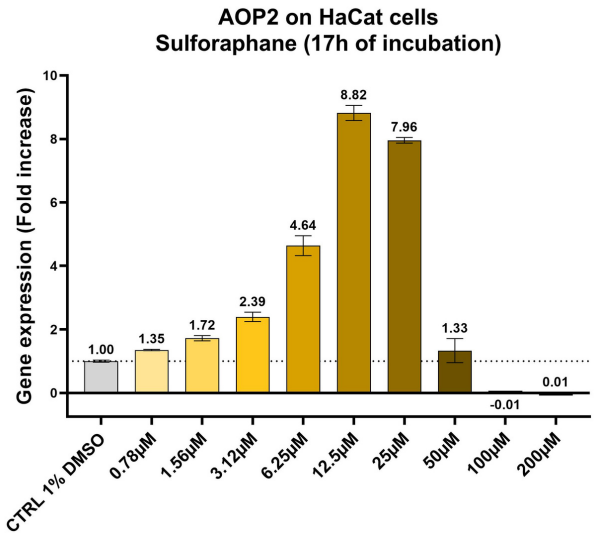
ASSAY FORMAT

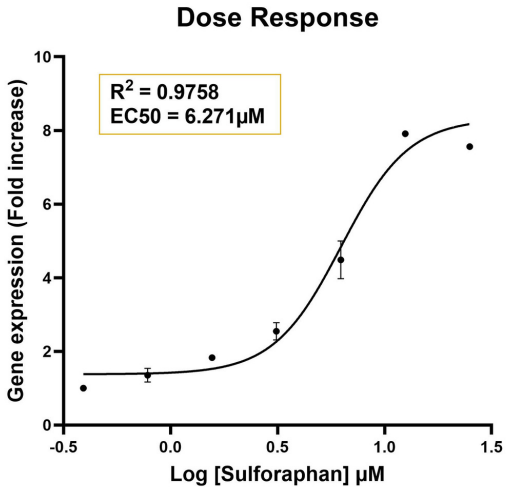
96-well cell culture plates

CELL MODEL

Human immortalized keratinocytes (HaCat)







AOP3 SKIN

Measure of antioxidant activity at the cell plasma membrane on human skin cells

ANTI OXIDANT POWER

ASSAY TYPE

Cell-based efficacy assay

SPECIFICITY

Evaluation of antioxidant activity by direct measurement of neutralization of peroxyl radicals at the cell plasma membrane

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 quercetine

ASSAY PRINCIPLE

Also known as CAA or DCFDA in the literature, this assay takes advantage of the presence of a diacetate (DA) group, which allows for the passage of DCFHDA across the plasma membrane. DCFHDA is cleavable by intracellular esterases, producing the non-permeable 2'-7'-dihydro-dichloro fluorescein (DCFH). When cells are treated with a radical generator such as 2,2'-azobis (2-amidinopropane) dihydro-chloride (AAPH), peroxyl radicals (ROO.) are produced at the plasma membrane level, triggering transformation of intracellular non-fluorescent DCFH into fluorescent dichloro-fluorescein (DCF). Consequently, a decrease in cellular fluorescence in AAPH-treated cells indicates an antioxidant effect of the sample at the level of ROOs. Kinetic records allow for antioxidant index calculation. Dose-response curves fitting with sigmoid model allow for evaluation of efficacy standard concentrations (EC10, EC50, EC90). Quercetine is used as a positive control.

DETECTION METHOD

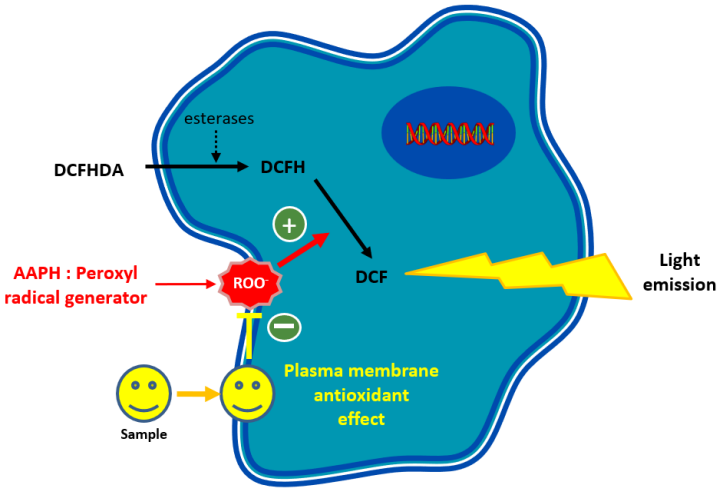
Fluorescence (exc/em 480-530 nm)

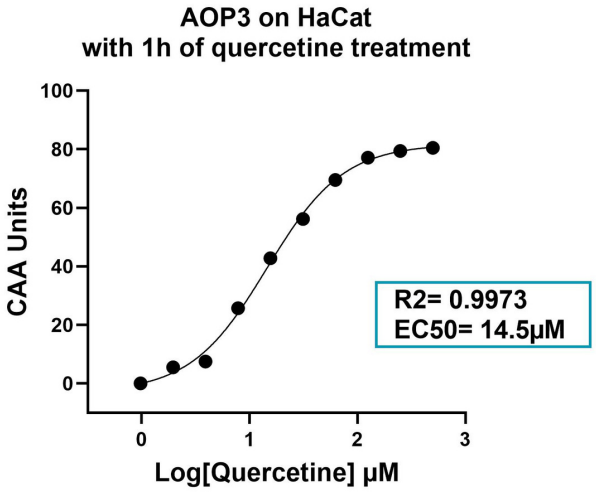
ASSAY FORMAT

96-well cell culture plates

CELL MODEL

Human immortalized (HaCaT) or primary keratinocytes, dermal fibroblasts, or other cell types.





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AOPCAT SKIN

Measure of CATALASE-like activity (H2O2 neutralization) in human skin cells

ANTI OXIDANT POWER

ASSAY TYPE

Cell-based efficacy assay

SPECIFICITY

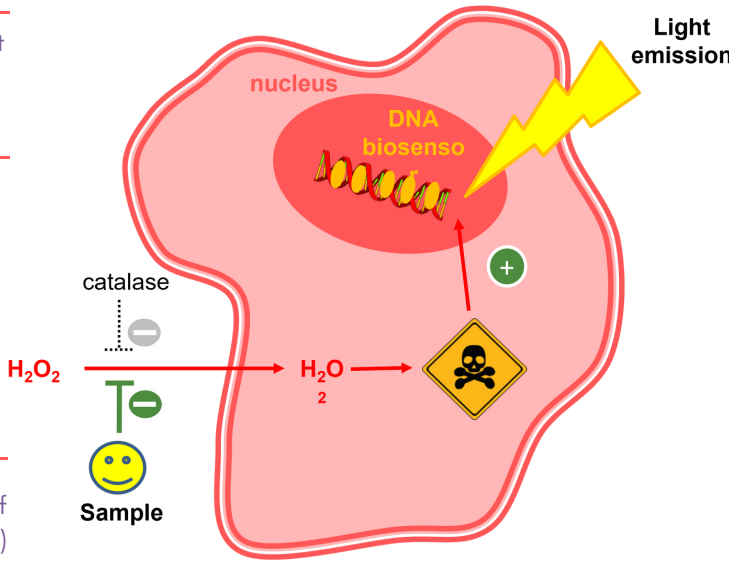
Evaluation of antioxidant catalase-like activity in human skin cells by direct measurement of hydrogen peroxide neutralization capacity of the sample.

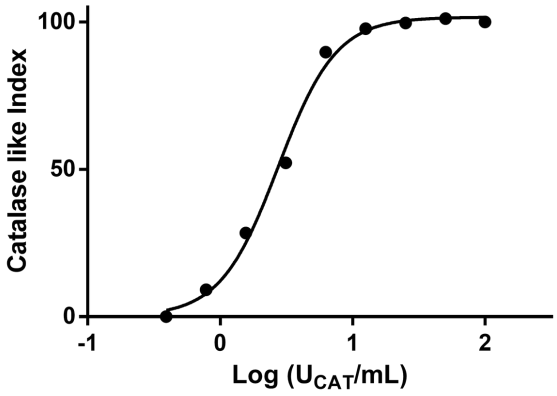
STUDY SPECIFICATIONS

- 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 EUK134 antioxidant

ASSAY PRINCIPLE

Reactive Oxygen Species (ROS) and free radicals are usually byproducts of the dioxygen reduction to water. This can happen within (mitochondria) and outside the cells. In living organisms, specific enzymes such as SOD and CAT catalyse this reduction, CAT being responsible of reduction of hydrogen peroxide (H2O2) to water. The assay is based on the intracellular presence of a DNA biosensor whose fluorescence increases in presence of H2O2. Decrease or time delayed increase of fluorescence indicates capacity of sample to neutralize H2O2 in a catalase-like reaction. Well-known antioxidants such as EUK134 for instance present this activity. Kinetic records allow for antioxidant index calculation. Dose-response curves fitting with sigmoid model allow for evaluation of efficacy standard concentrations (EC10, EC50, EC90) (patented technology). Bovine liver catalase provides a positive control.





DETECTION METHOD

Fluorescence (exc/em 505-535 nm)

ASSAY FORMAT

96-well cell culture plates

CELL MODEL

Human immortalized (HaCaT) or primary keratinocytes, dermal fibroblasts, or other cell types.

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AOP POL SKIN

Anti-pollution assay by cellular antioxidant activity detection

ANTI OXIDANT POWER

ASSAY TYPE

Cell-based efficacy assay

SPECIFICITY

Evaluation of anti-pollution activity by direct measurement of neutralization of oxidative stress induced by urban dust particules .

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 N-acetylcysteine

ASSAY PRINCIPLE

The assay is based on the DCFDA (or CAA) method. The DCFHDA probe is cleavable by intracellular esterases, producing the non-permeable 2'-7'-dihydro-dichloro fluorescein (DCFH). When cells are treated with an urban dust particle mix (Standard Reference Material® 1649b, National Institute of Standards & Technology) it induces an oxidative stress that triggers the transformation of intracellular non-fluorescent DCFH into fluorescent dichloro-fluorescein (DCF). Consequently, a decrease in cellular fluorescence in pollution-exposed cells indicates an anti-pollution effect of the sample by its capacity to attenuate the pollution-induced oxidative stress. Kinetic records allow for an Anti-POllution index (APO index) calculation. Dose-response curves fitting with sigmoid model allow for evaluation of efficacy standard concentrations (EC10, EC50, EC90). N-acetylcysteine (NAC) is used as a positive control.

DETECTION METHOD

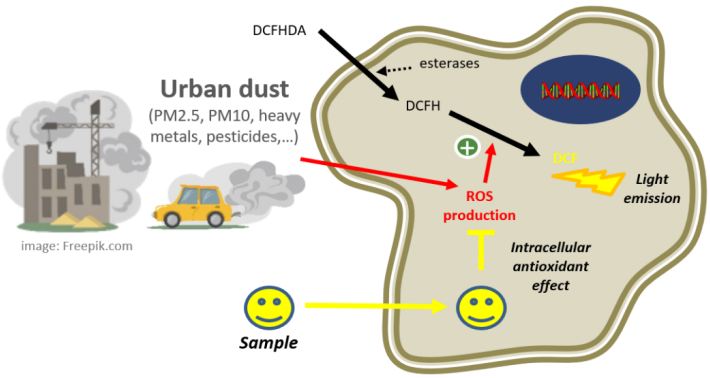
Fluorescence (exc/em 480-530 nm)

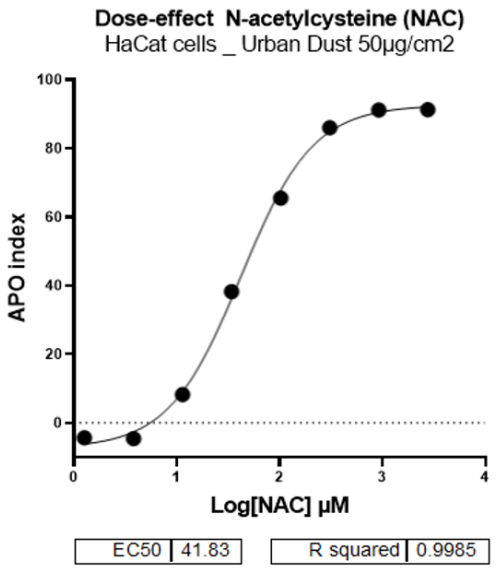
ASSAY FORMAT

96-well cell culture plates

CELL MODEL

Immortalized keratinocytes (HaCaT). Other cell models could be tested on request.





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AOP SKIN UVA

Measure of anti-UVA effect in human skin cells

ANTI OXIDANT POWER

ASSAY TYPE

Cell-based efficacy assay

SPECIFICITY

Evaluation of the effect by measurement of the neutralization of the cytotoxicity induced by UVA.

STUDY SPECIFICATIONS

- Full dose-effect study (7 doses)
- Calculation of a cell viability index for each dose after UVA exposure
- Evaluation of viability improvement
- At least two independent experiments
- Three end-point measurements (triplicate) for each dose
- Analytical report and monographs

ASSAY PRINCIPLE

The ultraviolet (UV) portion of the sun's radiation can permanently affect skin tissue. It contributes to skin aging and can induce various skin diseases, including inflammation, degenerative aging and cancer. The longest and most abundant UV rays are UVA (320-400 nm). They can penetrate deep into the dermis and induce the formation of intracellular reactive oxygen species (ROS), leading to oxidative damage in the cells of the epidermis and dermis.

In the SKIN UVA assay, epidermal cells in culture are treated 1h with the product/extract of interest and expose to a dose of UVA that recreates 30 minutes of exposure under the sun of Malaga (Spain) in July (1). The consequences of the UVA exposure on cell viability are revealed 24h after irradiation by a resazurin assay. The reduction of resazurin (non fluorescent dye) in resorufin (fluorescent) reveals the metabolic activity of the living cells. This measure allows for viability improvement calculation (% of protection against UVA exposure in presence of test sample versus absence of test sample) .

DETECTION METHOD

Fluorescence (exc/em 560-590 nm)

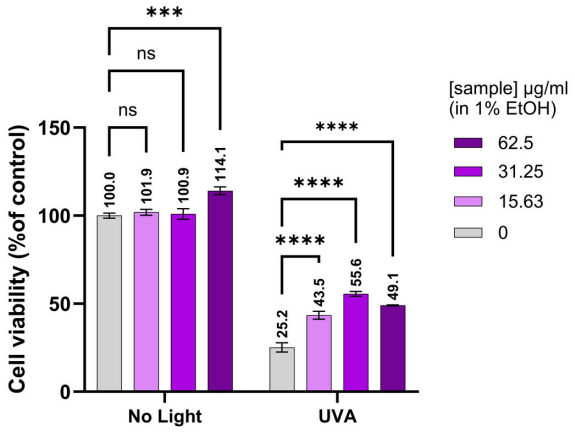
ASSAY FORMAT

96-well cell culture plates

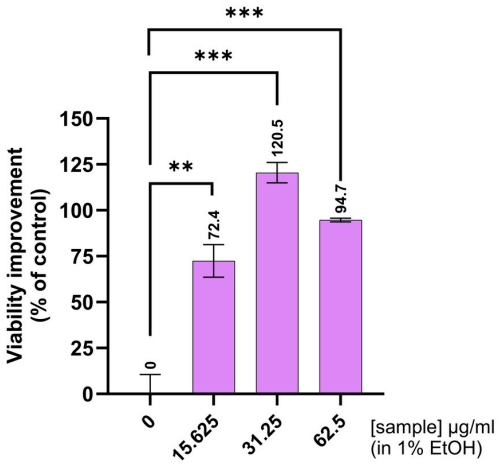
CELL MODEL

Human immortalized keratinocytes (HaCat)

Effect of a Resveratrol treatment on keratinocytes viability 24h after UVA exposure *(2way ANOVA analysis)*



Effect of a Resveratrol treatment on keratinocytes viability 24h after UVA exposure *(one-way ANOVA analysis)*



1. Gálvez, E. N. et al. The potential role of UV and blue light from the sun, artificial lighting, and electronic devices in melanogenesis and oxidative stress. J Photochem Photobiol B 228 (2022).

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