AOP IMM

Evaluation of immune modulatory effect

ANTI OXIDANT POWER

ASSAY TYPE

Cell-based efficacy assay

SPECIFICITY

Evaluation of immune modulatory effect of test compound through characterization of cytokine production by PBMCs

STUDY SPECIFICATIONS

- Dose effect study (3 doses)
- Viability assessment
- Determination of IL-6 interleukin production
- Two end-point measurements (duplicate) for each dose
- Analytical report and monographs

ASSAY PRINCIPLE

Human peripheral blood mononuclear cells (PBMCs) are used to investigate the effect of food bioactives on various immune cells. PBMCs are isolated from peripheral blood of donors. Identified as any blood cell with a round nucleus, they are typically composed of lymphocytes, monocytes and rare dendritic cells. Most of these cells are "naïve" or resting cells. Immune responses are stimulated by exposure to antigens. Upon stimulation, the cells enter a differentiation program, develop different effector functions and produce cytokines. Microbial lipopolysaccharide (LPS) is used for in vitro stimulation. PBMCs are incubated with a serial dilution of the test sample, in presence or absence of LPS. Quantification of the cytokine profile may provide information whether the test sample exerts a pro-inflammatory or anti-inflammatory effect on the immune response of PBMCs. Investigation of the cytokine profile is carried out by ELISA analysis of secreted cytokines in the culture supernatant.

DETECTION METHOD

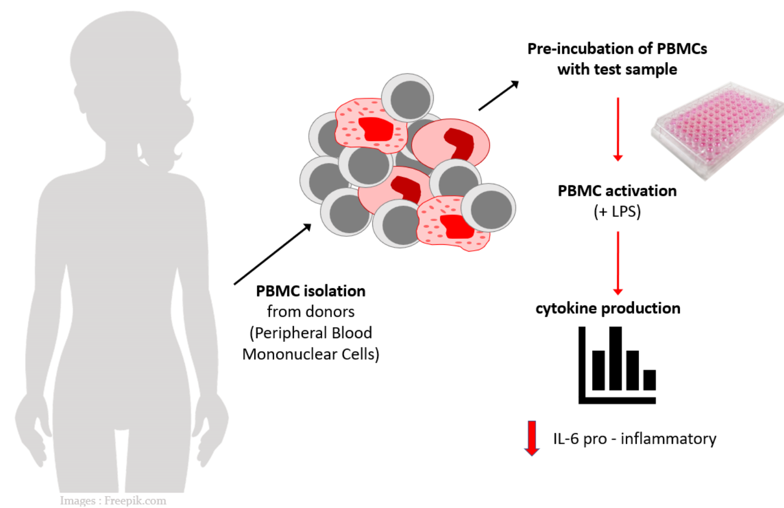
ELISA assay (Enzyme-Linked ImmunoSorbent Assay) on PBMC supernatant

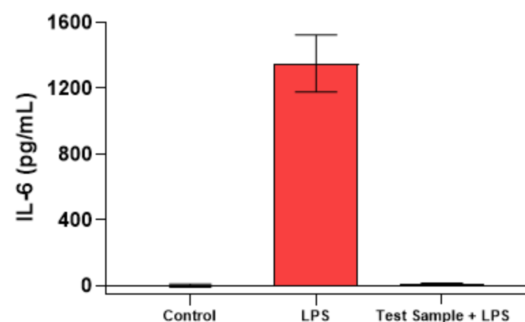
ASSAY FORMAT

96-well cell culture plates

CELL MODEL

Peripheral Blood Mononuclear Cells (PBMCs)





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AOP TOX

Measure of cytotoxicity/viability

ASSAY TYPE

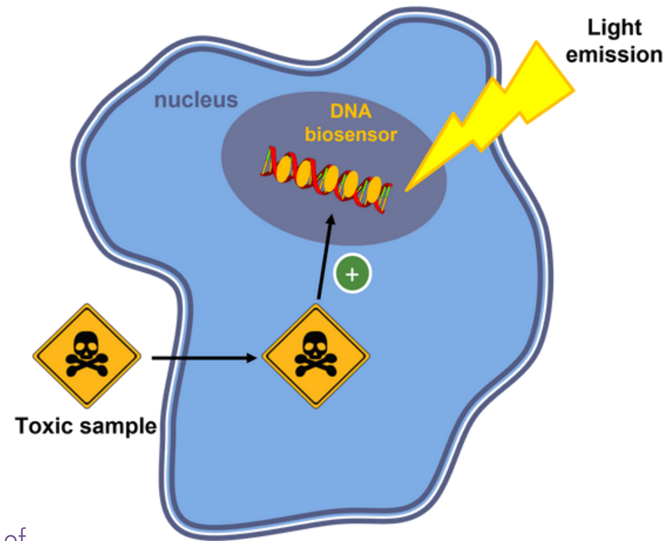
Cell-based efficacy assay

SPECIFICITY

Evaluation of acute (24h) toxicity/viabillity by measurement of loss of cell homeostasis

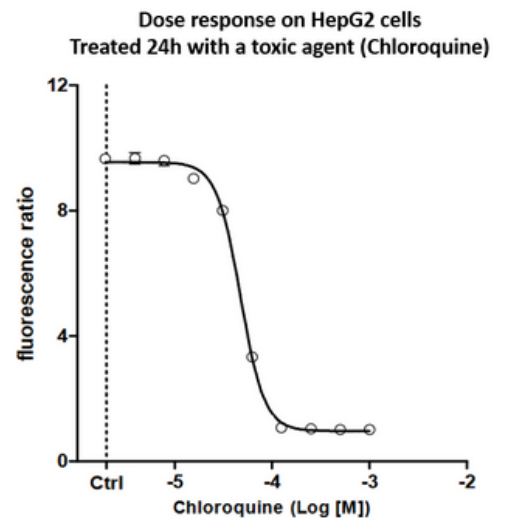
STUDY SPECIFICATIONS

- 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 cytotoxic agent chloroquine



ASSAY PRINCIPLE

The patented LUCS technology 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 fluorescent marker in the culture medium and two fluorescent measurements. The LUCS technology owned by AOP is based on photo-induction of a biosensor leading to a fluorescence signal. This fluorescence increase is only observed on cells in homeostasis prior to biosensor addition. Calculation of the fluorescence ratios before and after LED application leads to an accurate and dose-dependent measurement of the state of cellular damage that follows chemical or physical toxicity induced by the sample. The cellular and molecular mechanisms explaining the LUCS as well as the validation of the applications of the toxicology test have been published (1).
(**patented technology**).



DETECTION METHOD

Fluorescence (exc/em 505-535 nm)

ASSAY FORMAT

96-well cell culture plates

CELL MODEL

Human immortalized hepatocytes (HepG2) or other cell types.

(1) Derik et al: "Light-Up Cell System (LUCS), a universal high throughput assay for homeostasis evaluation in live cells", published in 2017 Scientific Reports, the online version of Nature (https://www.nature.com/articles/s41598-017-18211-2.pdf)

AOP TRANS

Assessment of intestinal absorption

ASSAY TYPE

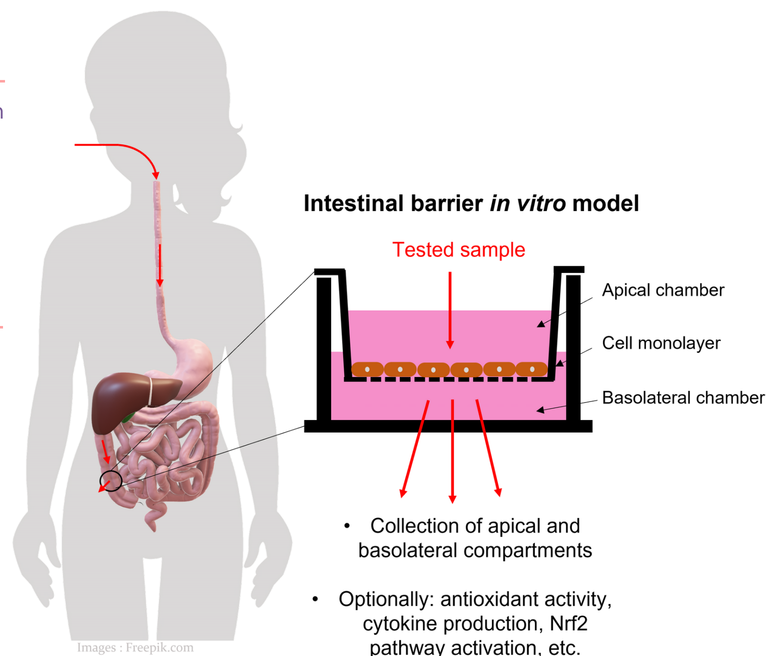
Cell-based efficacy assay

SPECIFICITY

Evaluation of intestinal absorption or trans-epithelial transport through enterocyte-like model

STUDY SPECIFICATIONS

- Two doses of test compound loaded on intestinal cell monolayers in apical chambers of transwell inserts
- Evaluation of Trans Epithelial Electric Resistance (TEER)
- Collection of basolateral and apical compartments
- Analytical report and monographs



ASSAY PRINCIPLE

Intestinal epithelium functions as a physical barrier between the contents of the gut lumen and internal milieu of our body, and is responsible for efficient absorption and uptake of essential nutrients. *In vitro* - differentiated human epithelial cell monolayers allow for testing of absorption and active and passive transports through the intestinal epithelium (1). The read-out parameter of the study is effect of the test sample on the epithelial barrier function, i. e. absorption or transepithelial transport. As the barrier function depends on the uniformity and integrity of the confluent and polarized cell monolayer cultivated on the permeable support, intestinal cells monolayer integrity is verified by measuring transepithelial electric resistance (TEER). After absorption, the apical and basolateral compartments are collected for further experiments. In option, theses fractions could be tested on different human cells as target organs, for direct or indirect antioxidant activity testing (AOP1, AOP2, AOP3 tests) (2), or anti-inflammatory cytokine production (AOP IMM test on human peripheral blood cells).

VALIDATION METHOD

Cytotoxicity assessment (LUCS)
TEER (Measure of resistance in ohms (Ω) x cm2)

ASSAY FORMAT

12-well cell culture plates

CELL MODEL

intestinal cells

(1): Hubatsch I, Ragnarsson EG, Artursson P. Determination of drug permeability and prediction of drug absorption in Caco-2 monolayers. Nat Protoc. 2007;2(9):2111-9.

(2): Furger C, Gironde C, Rigal M, Dufour C, Guillemet D. Cell-Based Antioxidant Properties and Synergistic Effects of Natural Plant and Algal Extracts Pre and Post Intestinal Barrier Transport. Antioxidants (Basel). 2022 Mar 16;11(3):565.

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AOP1

Measure of intracellular radical scavengin activity in human hepatocytes

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

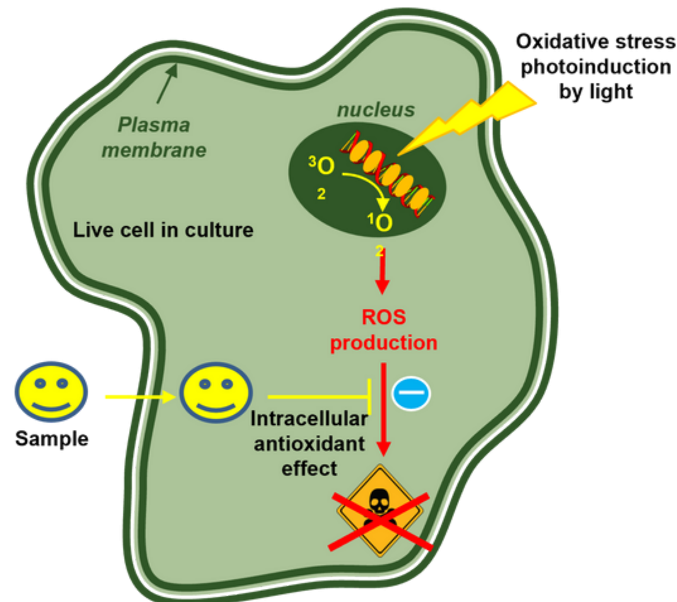
Fluorescence (exc/em 505-535 nm)

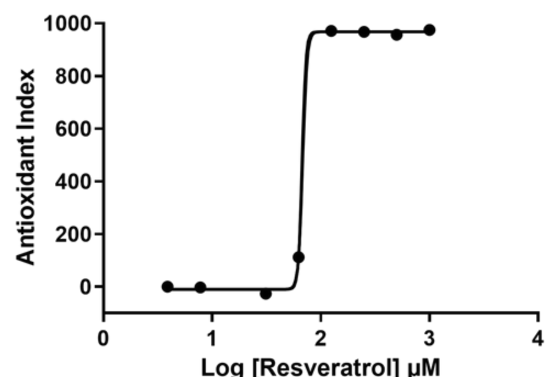
ASSAY FORMAT

96-well cell culture plates

CELL MODEL

Human immortalized hepatocytes (HepG2) or other cell models (enterocyte-like, neuron-like, keratinocytes, fibroblasts, etc.)





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AOP2

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

ASSAY TYPE

Cell-based efficacy assay

SPECIFICITY

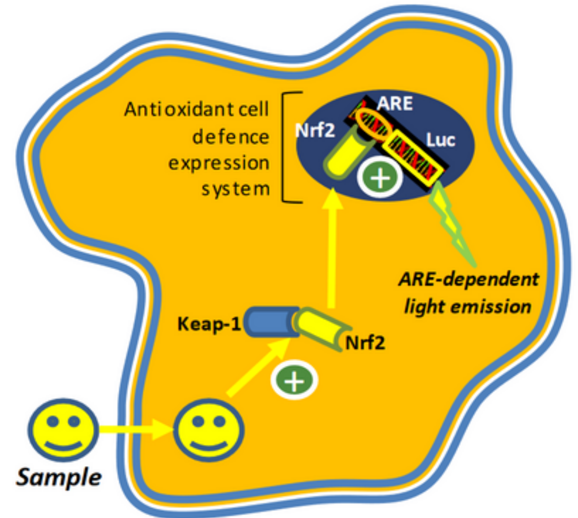
Evaluation of natural cell defence by Antioxidant Responsive Element (ARE) induction through Nrf2 transcription factor

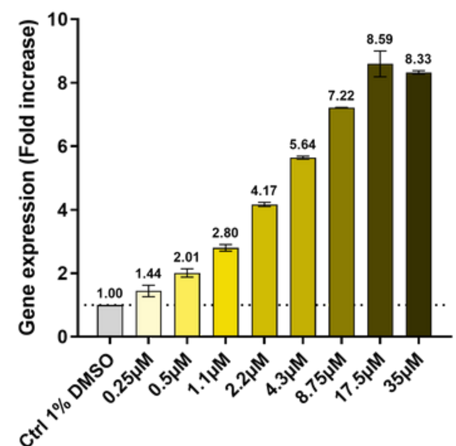
STUDY SPECIFICATIONS

- 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

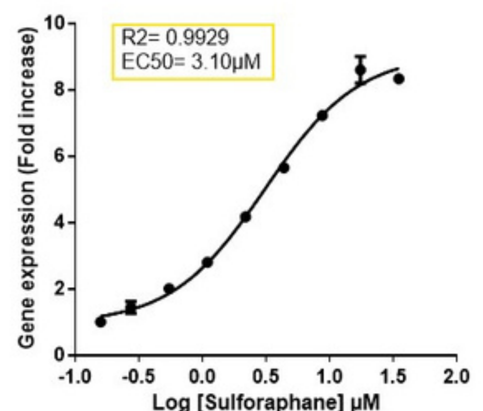
Luminescence

ASSAY FORMAT

96-well cell culture plates

CELL MODEL

Human immortalized hepatocytes (HepG2)



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AOP3

Measure of antioxidant activity
at the cell plasma membrane
on human hepatocytes

ASSAY TYPE

Cell-based efficacy assay

SPECIFICITY

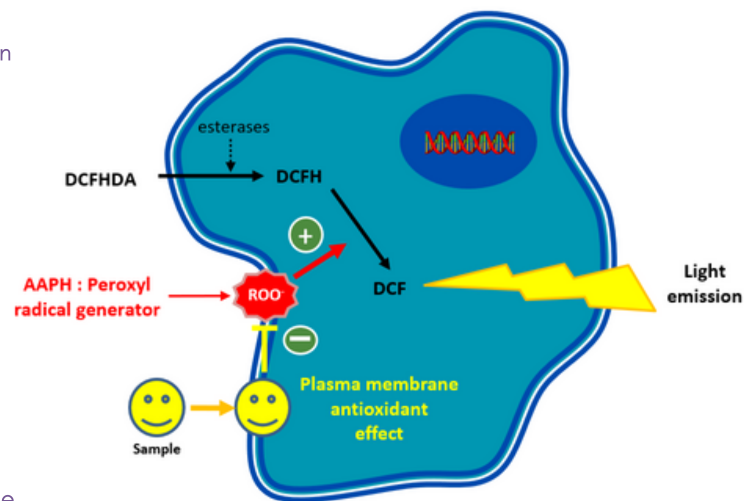
Evaluation of antioxidant activity by direct measurement of neutralization of peroxyl radicals at the cell plasma membrane

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

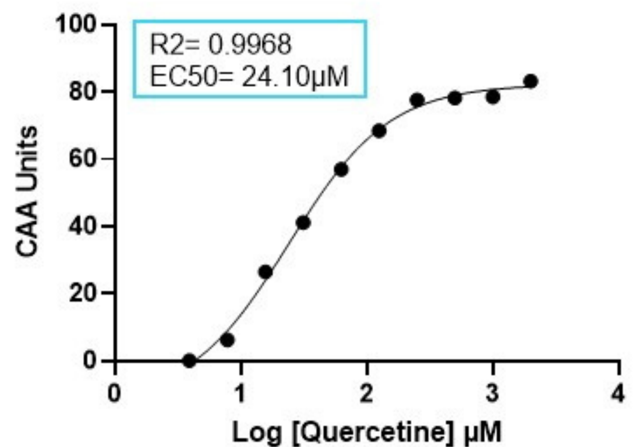
ASSAY PRINCIPLE

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 hepatocytes (HepG2) or other cell models (enterocyte-like, neuron-like, keratinocytes, fibroblasts, etc.)

AOPCAT

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

ASSAY TYPE

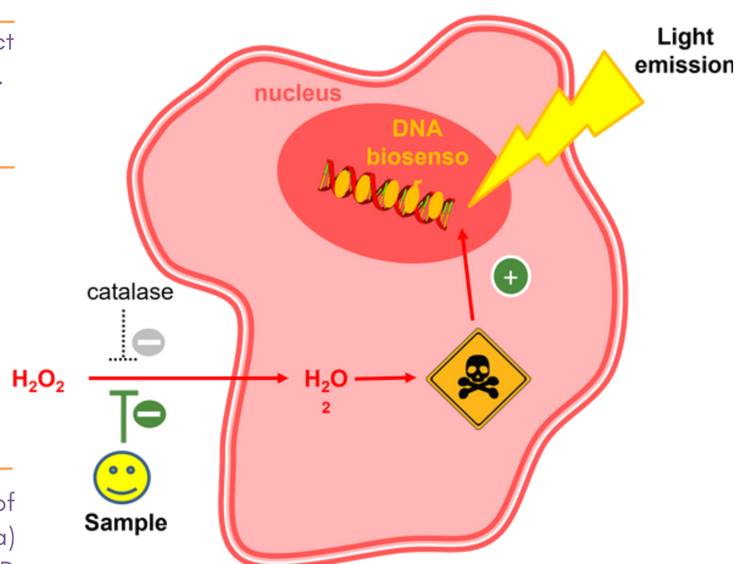
Cell-based efficacy assay

SPECIFICITY

Evaluation of catalase-like activity on human hepatocytes by direct measurement of hydrogen peroxide neutralization capacity of the sample.

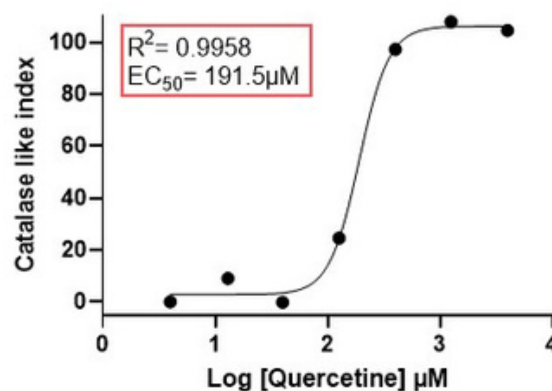
STUDY SPECIFICATIONS

- 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 hepatocytes (HepG2) or other cell models (enterocyte-like, neuron-like, keratinocytes, fibroblasts, etc.)

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