

AOP1 HAIR

Measure of intracellular antioxidant activity in human primary hair cells



ASSAY TYPE

Cell-based efficacy assay

SPECIFICITY

Evaluation of antioxidant activity in human primary hair cells 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 in human primary hair cell. 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 ($^3\text{O}_2$) resulting in the production of singlet oxygen ($^1\text{O}_2$) which in turn triggers a cascade of Reactive Oxygen Species (ROS) production including the free radical species superoxide anion ($\text{O}_2^{\bullet-}$) and hydroxyl radical ($\text{OH}^{\bullet}$). 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 and EC90) evaluation (**patented technology**).

DETECTION METHOD

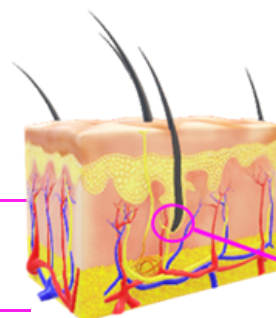
Fluorescence (exc/em 505-535 nm)

ASSAY FORMAT

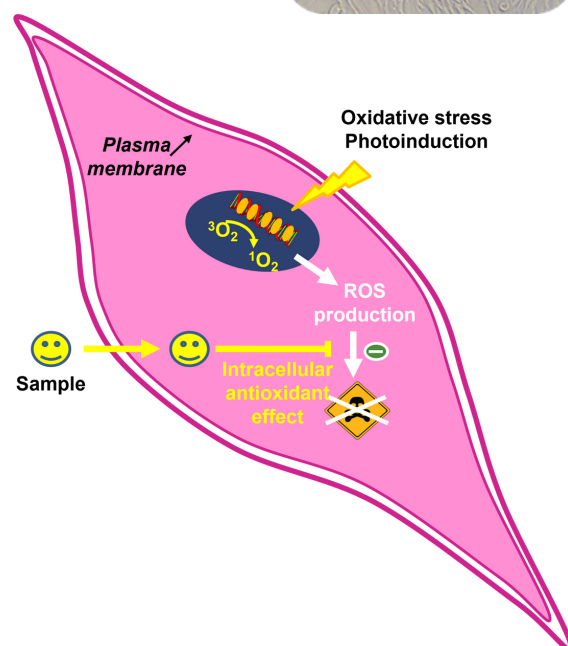
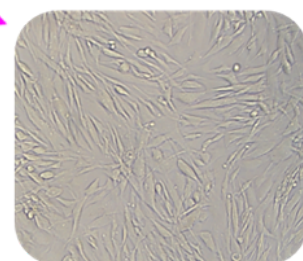
96-well cell culture plates

CELL MODEL

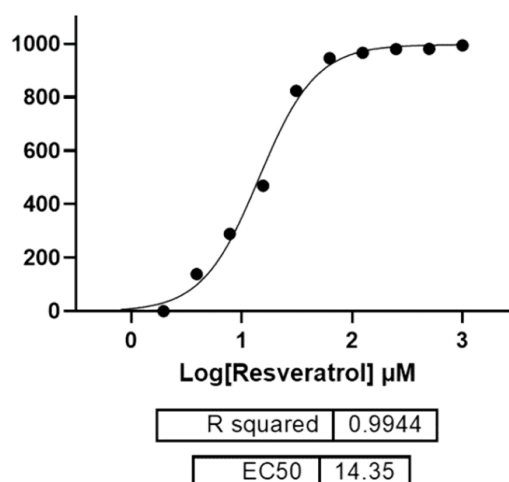
Primary Human Follicle Dermal Papilla Cells (HFDPC, Female, Caucasian, with light pigmentation).
Other donor cells upon request.



Human Dermal Papilla Cells
in 96 well plate



Dose-effect Resveratrol
Human Follicle Dermal Papilla Cells



AOP3 HAIR

Measure of antioxidant activity at the cell plasma membrane in human primary hair cells



ASSAY TYPE

Cell-based efficacy assay

SPECIFICITY

Evaluation of antioxidant activity in human primary hair cells by direct measurement of neutralization of peroxy 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 standard antioxidant 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), peroxy 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 CAA units calculation. Dose-response curves fitting with sigmoid model allow for evaluation of efficacy standard concentrations (EC10, EC50, EC90).

DETECTION METHOD

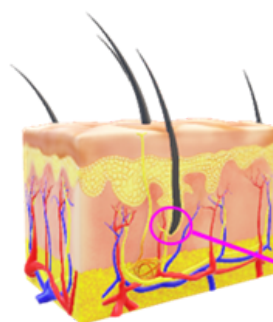
Fluorescence (exc/em 480-530 nm)

ASSAY FORMAT

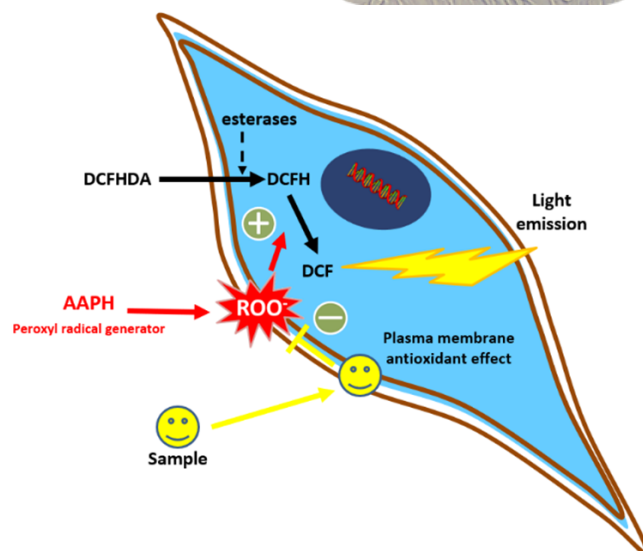
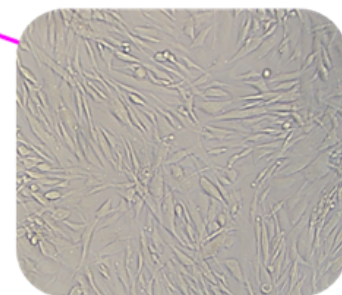
96-well cell culture plates

CELL MODEL

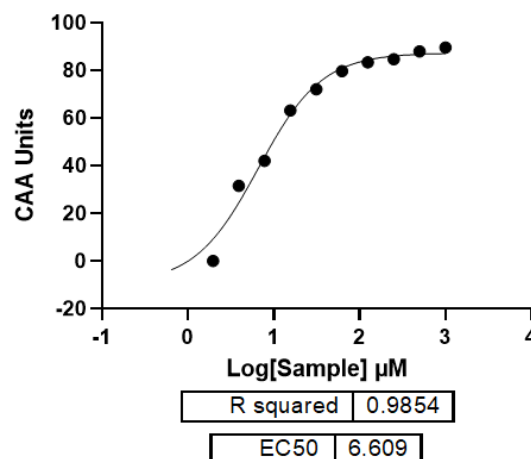
Primary Human Follicle Dermal Papilla Cells (HFDPC, Female, Caucasian, with light pigmentation).
Other donor cells upon request.



Human Dermal Papilla Cells
in 96 well plate



Dose-effect Quercetin
Human Follicle Dermal Papilla Cells



AOPCAT HAIR

Measure of CATALASE-like activity (H_2O_2 neutralization) in human primary hair cells



ASSAY TYPE

Cell-based efficacy assay

SPECIFICITY

Evaluation of antioxidant catalase-like activity in human primary hair cells by direct measurement of hydrogen peroxide scavenging 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 (H_2O_2) to water. The assay is based on the intracellular presence of a DNA biosensor whose fluorescence increases in presence of H_2O_2 . Decrease or time delayed increase of fluorescence indicates capacity of sample to neutralize H_2O_2 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

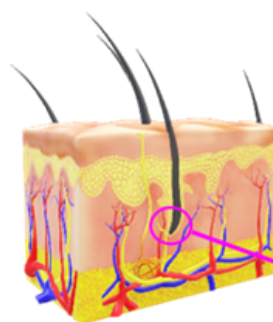
Fluorescence (exc/em 505-535 nm)

ASSAY FORMAT

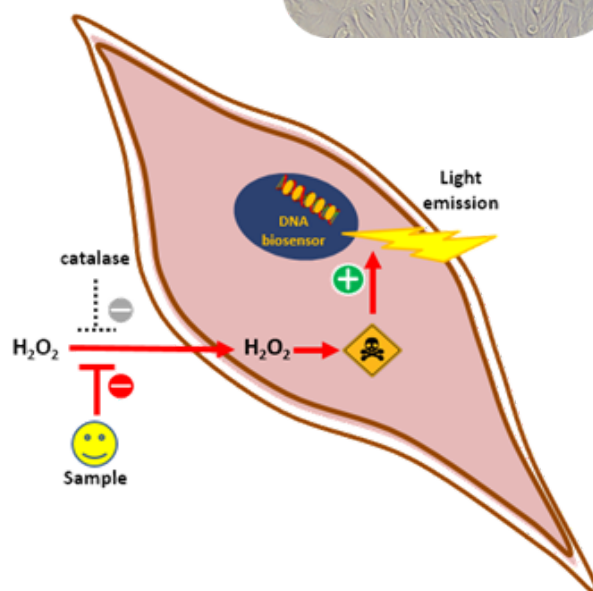
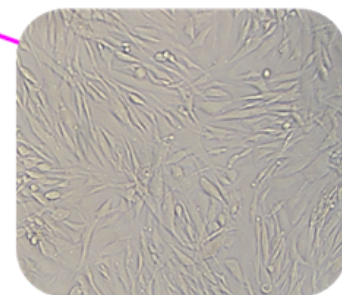
96-well cell culture plates

CELL MODEL

Primary Human Follicle Dermal Papilla Cells (HFDPC, Female, Caucasian, with light pigmentation). Other donor cells upon request.



Human Dermal Papilla Cells in 96 well plate



Dose-effect of EUK134 Human Follicle Dermal Papilla cells

