

# AOP3 SKIN

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



## ASSAY TYPE

Cell-based efficacy assay

## SPECIFICITY

Evaluation of antioxidant activity 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 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 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

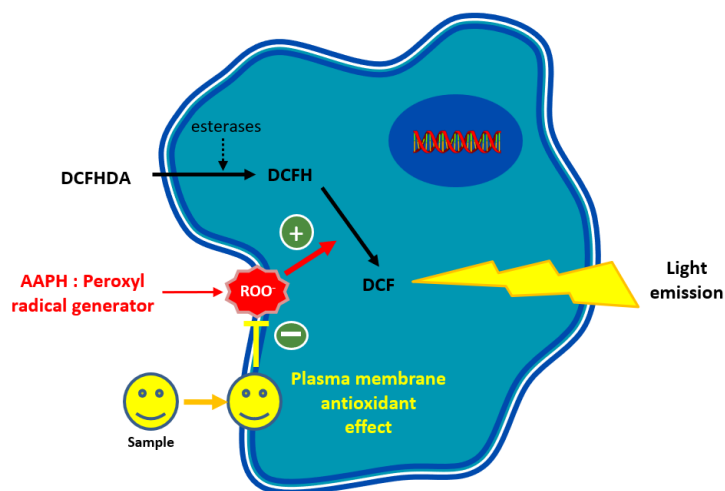
Fluorescence (exc/em 480-530 nm)

## ASSAY FORMAT

96-well cell culture plates

## CELL MODEL

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



AOP3 on HaCat  
with 1h of quercetine treatment

