

AOP POL SKIN

Anti-pollution assay by cellular antioxidant activity detection



ASSAY TYPE

Cell-based efficacy assay

SPECIFICITY

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

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.

