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)