

Objective

To identify compounds which can prevent free radical buildup and/or treat oxidative stress inducers, Mattek's Normal Human Epidermal Keratinocytes (NHEKs) were used to screen potential antioxidants.

Methods

Mattek's Adult NHEKs (NHEK-CRY-AD) were cultured in NHEK Growth Medium (NHEK-GM) according to manufacturer's protocol (Figure 1). Cells were pre-treated with increasing concentrations of potential antioxidants for 24 hours. Cells were incubated with 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) for 1 hour. 25 μ L of each formulation was applied topically for 24 hrs. Intracellular ROS was induced by a 1 hour incubation with 500 μ M H₂O₂. Levels of 2',7'-dichlorodihydrofluorescein (DCF), generated by ROS, were measured and changes in ROS signal were compared to control (Figure 2).

Note: Accumulation of reactive oxygen species (ROS) in epidermal keratinocytes can cause oxidative stress and premature skin aging.



Figure 1. Mattek's Adult Normal Human Epidermal Keratinocytes (NHEK-CRY-AD), 10x Magnification.

Results

Adult NHEKs treated with either Antioxidant 1 or Antioxidant 2 showed dose-dependent decreases in intracellular reactive oxygen species following induction by H₂O₂ (Figure 2).

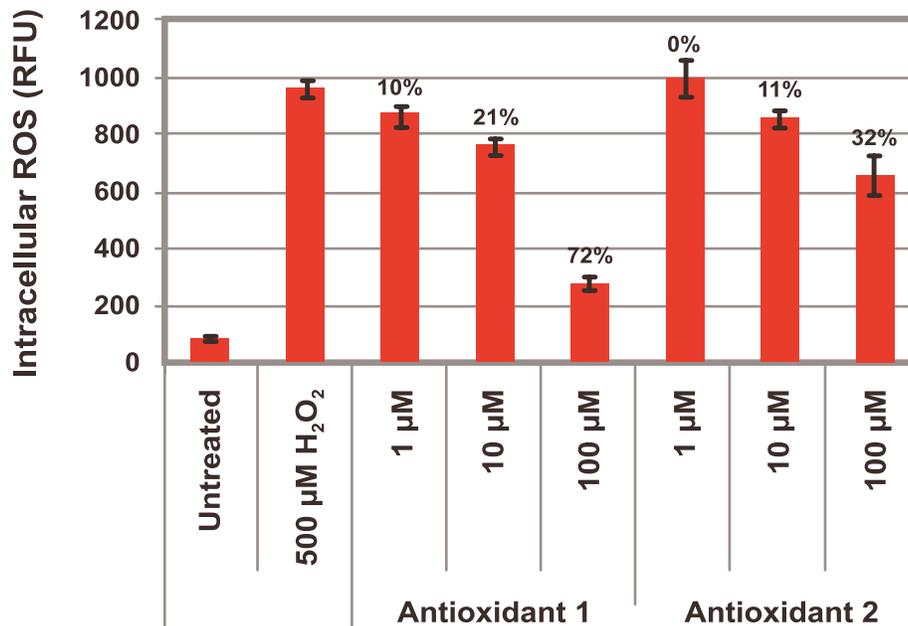


Figure 2. Mattek's Adult Normal Human Epidermal Keratinocytes (NHEK-CRY-AD) were treated with increasing concentrations of antioxidant test compounds for 24 hours and then exposed to 500 μ M H₂O₂. Significant decreases in intracellular ROS levels were observed with increased concentrations of Antioxidant 1 and Antioxidant 2. Percent decrease compared to control indicated above treatments.

Conclusion

Mattek's Normal Human Epidermal Keratinocytes can be used to identify antioxidant compounds for personal care and cosmetic product development.