DPPH Antioxidant Assay

ACTIVE CONCEPTS LLC
107 Technology Drive, Lincolnton | NC 28092 USA
• info@activeconceptsllc.com • Phone: +1-704-276-7100 • Fax: +1-704-276-7101 • https://activeconceptsllc.com/

Tradename: AC OleaShield

Code: 12006

CAS #: 68333-16-4 (or) 92128-79-5 & 8001-25-0 & 68333-16-4 (or) 1686112-36-6 (or) 9015-54-7

Test Request Form #: 10183

Lot #: N230120A

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Drumwright*
Principle Investigator: *Daniel Shill*

Test Performed:
DPPH (2,2-diphenyl-1-picrylhydrazyl) Antioxidant Assay

Introduction

Reactive oxygen species (ROS) and free radicals are generated by normal cellular processes, aging, pollutants, foreign substances, and UV irradiation. Large and unregulated increases in ROS/free radicals accelerate DNA mutation, cellular senescence, advanced glycation end products, protein oxidation, and collagen degradation. When intrinsic antioxidant capacities are reduced, such as during aging, the imbalance between pro- and anti-oxidant systems further accentuates these hallmarks of cellular aging and quenching excessive free radicals through exogenous means becomes increasingly important. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a well-protected organic nitrogen radical that provides a quick assessment of a test substance's potential antioxidant capacity through electron and hydrogen atom transfer.

Accordingly, a DPPH Antioxidant Assay was conducted to assess the radical scavenging activity of **AC OleaShield**. Attenuating excessive radicals preserves cellular homeostasis and blunts intrinsic and extrinsic age-related declines in antioxidant capacity.

Assay Principle

The DPPH free radical is a long-lived and well-protected organic nitrogen radical. When an antioxidant encounters DPPH, the color shifts from a deep purple to yellow in proportion to the radical scavenging capacity of the substance which is evaluated by monitoring the decrease of its absorbance at 517 nm.

ACTIVE CONCEPTS LLC
107 Technology Drive, Lincolnton | NC 28092 USA
• info@activeconceptsllc.com • Phone: +1-704-276-7100 • Fax: +1-704-276-7101 • https://activeconceptsllc.com/

Materials

A. Incubation Conditions:	Room Temperature (~25°C) and Dark
B. Equipment:	Synergy HT Microplate Reader; Pipettes; Analytical Balance
C. Reagents/Buffers:	DPPH (2,2-Diphenyl-1-picrylhydrazyl) (Millipore-Sigma; D9132)*; 1 M Tris-HCl Buffer (pH 7.5) (ThermoFisher, 15567027)*; Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) (Millipore-Sigma; 238813)*; Ethanol
D. Culture Plate:	96 Well Flat and Clear Bottom Tissue Culture Microplate
E. Other:	Sterile disposable pipette tips

*Or suitable alternatives, subject to change without notice based off vendor availability

Methods

Stock solutions of DPPH, Trolox, and **AC OleaShield** were prepared in ethanol. 100 µL of 0.2 mM DPPH was added to 80 µL Tris-HCl Buffer and the final 20 µL added was comprised of the test substance, Trolox, or ethanol. **AC OleaShield** was tested at final well concentrations of 1.25%, 2.5%, 5.0%, and 10.0%. For the DPPH Control, 20 µL of ethanol was added instead of Trolox or the test substance. After a 30-minute incubation in the dark at room temperature, the absorbance (Abs) was measured at 517 nm.

The radical scavenging activity (RSA) of each sample was calculated by the following equation:

$$Radical\ Scavenging\ Activity\ (RSA)\ (\%) = \frac{Abs_{DPPH\ Control} - Abs_{Sample}}{Abs_{DPPH\ Control}} \times 100$$

Results

The data obtained from this study met criteria for a valid assay and the controls performed as anticipated. **AC OleaShield** demonstrated potent radical scavenging activity.

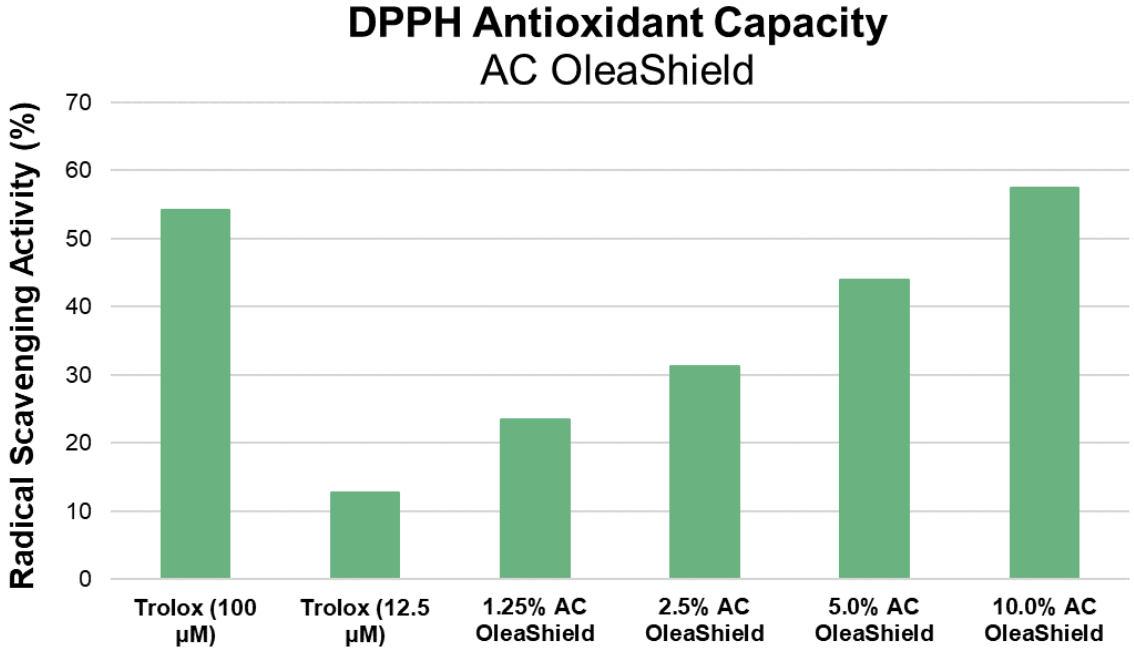

Figure 1. The radical scavenging activity of **AC OleaShield**.

ACTIVE CONCEPTS LLC
107 Technology Drive, Lincolnton | NC 28092 USA
• info@activeconceptsllc.com • Phone: +1-704-276-7100 • Fax: +1-704-276-7101 • https://activeconceptsllc.com/

Discussion

As shown in Figure 1, **AC OleaShield** exhibited greater antioxidant activity than 12.5 µM Trolox. The radical scavenging activity of **AC OleaShield** increased as the concentration increased, indicating the antioxidant properties of **AC OleaShield** are dose dependent. Provided the nitrogen radical of DPPH is well-protected, these results demonstrate the strong ability of **AC OleaShield** to scavenge harmful radicals that are not easily reduced.

Collectively, quenching excessive radicals with exogenous assistance preserves cellular homeostasis and blunts intrinsic and extrinsic age-related declines in antioxidant capacity. These data indicate **AC OleaShield** is capable of scavenging well-protected radicals, which may help to attenuate characteristics of cellular aging.